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The present volume contains the contributions from the Woods Hole Oceanographic Institution which appeared during 1933 in publications other than *Papers in Physical Oceanography and Meteorology;* also the Annual Report of the Director.

TABLE OF CONTENTS

Contribution
No.

6. SELMAN A. WAKSMAN and CORNELIA L. CAREY
Rôle of Bacteria in Decomposition of Plant and Animal Residues in the Ocean

7. ARDA ALDEN GREEN and RAYMOND W. ROOT
The Equilibrium between Hemoglobin and Oxygen in the Blood of certain Fishes

8. NORRIS W. RAKESTRAW
Studies on the Biology and Chemistry of the Gulf of Maine. I. Chemistry of the waters of the Gulf of Maine in August, 1932

9. H. H. GRAN
Studies on the Biology and Chemistry of the Gulf of Maine. II. Distribution of phytoplankton in August, 1932

10. SELMAN A. WAKSMAN, H. W. REUSZER, CORNELIA L. CAREY, MARGARET HOTCHKISS, and C. E. RENN
Studies on the Biology and Chemistry of the Gulf of Maine. III. Bacteriological investigations of the sea water and marine bottoms

11. ARDA A. GREEN and ALFRED C. REDFIELD
On the Respiratory Function of the Blood of the Porpoise

12. SELMAN A. WAKSMAN
On the Distribution of Organic Matter in the Sea Bottom and the Chemical Nature and Origin of Marine Humus

13. ALFRED C. REDFIELD
The Evolution of the Respiratory Function of the Blood

14. C. O'D. ISELIN, II
Some Phases of Modern Deep-Sea Oceanography

15. E. MONROE HARWOOD and CHARLES F. BROOKS
Monthly Sequence of Sea-Surface Temperature on the New York–San Juan Steamship Route

16. ALFRED C. REDFIELD and ELIZABETH N. INGALLS
The Oxygen Dissociation Curves of some Bloods containing Hemocyanin

Contribution
No.

17. SELMAN A. WAKSMAN, CORNELIA L. CAREY, and HERBERT W. REUSZER
    Marine Bacteria and Their Rôle in the Cycle of Life in the Sea. I. Decomposition of marine plant and animal residues by bacteria

18. SELMAN A. WAKSMAN, MARGARET HOTCHKISS, and CORNELIA L. CAREY
    Marine Bacteria and Their Rôle in the Cycle of Life in the Sea. II. Bacteria concerned in the cycle of nitrogen in the sea

19. HERBERT W. REUSZER
    Marine Bacteria and Their Rôle in the Cycle of Life in the Sea. III. The distribution of bacteria in the ocean waters and muds about Cape Cod

20. JOHN COLMAN
    The Woods Hole Oceanographic Institution: Sonic Soundings, and an Account of a Winter Cruise to the Somers Islands

21. JOHN H. WELSH
    Light Intensity and the Extent of Activity of Locomotor Muscles as Opposed to Cilia

26. GEORGE L. CLARKE
    Observations on the Penetration of Daylight into Mid-Atlantic and Coastal Waters

27. C. O'D. ISELIN
    The Development of Our Conception of the Gulf Stream System

29. NORRIS W. RAKESTRAW and FRANK B. LUTZ
    Arsenic in Sea Water

31. PHILIP H. MITCHELL and NORRIS W. RAKESTRAW
    The Buffer Capacity of Sea Water

33. GEORGE L. CLARKE
    Diurnal Migration of Plankton in the Gulf of Maine and Its Correlation with Changes in Submarine Irradiation

37. G. H. PARKER
    The Color Changes of Elasmobranch Fishes

THE WOODS HOLE OCEANOGRAPHIC INSTITUTION
Report for the Years 1930-1932

Contribution No. 6

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1933, xxx, pp. 526-527

6554

Rôle of Bacteria in Decomposition of Plant and Animal Residues in the Ocean.

SELMAN A. WAKSMAN AND CORNELIA L. CAREY.

From the Woods Hole Oceanographic Institution and the New Jersey Agricultural Experiment Station.

A study has been made of the decomposition of marine zoöplankton and of certain marine algae by bacteria in sea water and in marine mud. The formation of carbon dioxide and the liberation of nitrogen in the form of ammonia were used as measures of the rate of decomposition. It was found that the chemical nature of the material has a very important influence upon the rapidity and nature of its decomposition.

Zoöplankton, consisting largely of copepods, and containing 7.9% total N on a dry basis, was found to decompose very rapidly, both in the water and in the mud. Nearly one-third of the total C was liberated as CO_2 within 19 days' incubation, while a large part of the N was liberated as ammonia. A marked difference, however, was observed in the case of the medium in which the decomposition took place, namely, although the amount of N liberated was about the same in the water and in the mud, viz., 40.3 and 38.5 mg., a considerably greater amount of CO_2 was evolved in the mud than in the water, viz., 101 vs. 64 mg., above controls. This difference may be due to the fact that while the nitrogenous constituents of the zoöplankton decompose alike in water and in mud, some of the nitrogen-poor or non-nitrogenous complexes are decomposed more rapidly in mud than in water, probably due to the presence in mud of specific bacteria capable of attacking them.

For the decomposition of the algal material, *Ulva lactuca* and *Fucus vesiculosus* were employed. The decomposition of these substances, also, was quite different in water and in mud media; in

Ulva, which contained 1.67% N in the total dry material, there was as much decomposition in water as in mud medium, as shown by the CO_2 liberated, viz., 83.4 and 101.8 mg. C. This was accompanied by the accumulation of small amounts of ammonia, viz., 5.2 and 4.1 mg., respectively, above controls. In the case of the Fucus, which contained only 0.86% N, there was very little decomposition in the water, while considerable decomposition took place in the mud, viz., 10.4 and 85.8 mg. C as CO_2, respectively. The reason for this is very easy to find, when one observes that no N was liberated as a result of the decomposition of the Fucus material by the bacteria. Since the N content of Fucus is very low, considerably less than is needed by the bacteria to bring about its decomposition, the amount of available N proved to be the limiting factor in the extent of decomposition of the Fucus by the bacteria. Since the decomposition of the humus in the mud results in the liberation of small amounts of N in an available form, the bacteria are thus enabled to bring about a considerably greater decomposition of the Fucus in the mud than in the water. This assumption was found to be correct, when, on adding small amounts of N in the form of ammonium salts or nitrates to the sea water, a great increase in the decomposition of the Fucus material took place. It is also possible that mud harbors certain bacteria which are not present in the water and which are able to decompose some of the specific carbohydrates present in the Fucus.

The nature of the organic residues in the sea may thus exert an important influence upon the amount of available N present at any one time in the sea water.

Contribution No. 7

Reprinted from BIOLOGICAL BULLETIN, Vol. LXIV, No. 3, June, 1933

THE EQUILIBRIUM BETWEEN HEMOGLOBIN AND OXYGEN IN THE BLOOD OF CERTAIN FISHES

ARDA ALDEN GREEN AND RAYMOND W. ROOT
THE WOODS HOLE OCEANOGRAPHIC INSTITUTION[1]

In studies made on the hemoglobin of certain teleost fishes Root (1931) found that carbon dioxide had a remarkable effect on the ability of the blood to combine with oxygen. As in the case of mammalian blood, the addition of acid decreases the amount of oxygen combined with the hemoglobin at any given oxygen tension, but in these fish bloods the addition of acid decreases also the total amount of oxygen that can be combined even when the oxygen tension is increased to that of air. As a consequence the suggestion was made that the prosthetic groups in the hemoglobin molecule were differentially affected by the presence of acid, some being inactivated entirely so that they no longer combined with oxygen. Since confirmation of this suggestion promised to throw new light on the theory of the equilibrium between oxygen and hemoglobin, the phenomenon has been examined in greater detail.

In the present study we have examined the blood of the tautog, *Tautoga onitis* Linnæus, the goosefish, *Lophius piscatorius* Linnæus, and the toadfish, *Opsanus tau* (Linnæus). We have confirmed the observation of Root and shown that the amount of oxygen combined is decreased in the presence of acid even though the oxygen tension is raised to one atmosphere. In addition, the oxygen dissociation curves have been studied throughout the range of hydrogen ion concentration in which the phenomenon is described. These curves are remarkable as to shape, especially those of the toadfish, which have a distinct undulatory character. Another characteristic is a change in the shape of the curves with a change in pH. In alkaline solutions the curves for the goosefish and for the tautog are sigmoid whereas in more acid solutions the curves are rectangular hyperbolæ. Thus in these teleost fishes we find certain unique characteristics exhibited by the oxygen dissociation curves. Such peculiarities must be accounted for by any acceptable general theory of the union of oxygen with hemoglobins.

METHODS

The blood of the goosefish was obtained from the bulbus or sinus venosus, and was removed from the animal shortly after it was taken from the commercial fish traps. On account of the low hemoglobin concentration of the blood of this fish it was customary to centrifuge the blood and draw off plasma equal to about one-third the total volume, before making analyses. The other fishes were maintained at the laboratory in tanks, and were bled from the gills by means of a hypodermic syringe. Lithium oxalate was used as an anticoagulant. Samples of blood were necessarily combined in any given species of fish and the reserve supply kept on ice. In most cases the blood was used within 12 hours. In the case of the goosefish it was possible to keep a sample of blood several days without the appearance of methemoglobin.

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[1] Contribution No. 7.

The usual methods of obtaining oxygen dissociation curves were employed as described by Dill (1928). Carbon dioxide and oxygen analyses on the blood were done simultaneously as described by Root (1931). The analysis of gas mixtures containing over 30 per cent carbon dioxide or oxygen was made possible by a special modification of the Haldane apparatus. Gas pressures in the tonometers were calculated by use of the formula of Bock, Field, and Adair (1924). Samples of blood were equilibrated at a temperature of 25° C. for 15 minutes in a water bath. Nitrogen was used as the inactive gas in the tonometers. It is essential to point out that but one tonometer was equilibrated at a time and analyses followed directly after equilibration. This procedure is quite necessary in fish blood, since it possesses active, nucleated erythrocytes (Dill, Edwards, and Florkin, 1932). Duplicate analyses, both of the gases in the blood and in the tonometer, were made for all established points.

Since the oxygen combined by these bloods is diminished in the presence of acid, the oxygen capacities were always determined on blood to which a small amount of powdered sodium bicarbonate had been added. This blood was then equilibrated either in air or in oxygen and the figure obtained after dissolved oxygen was subtracted was taken to be the real oxygen capacity of the hemoglobin.

A few of the more acid samples turned brownish in color and the formation of methemoglobin was suspected. In such cases the oxygen capacity of a duplicate sample of the blood was determined directly after each equilibration by using sodium bicarbonate to bring the blood once more to an alkaline reaction and re-equilibrating with air. Whenever the hemoglobin had been irreversibly inactivated the percentage saturation was calculated by using the oxygen capacity as determined after equilibration rather than the original value.

All the data expressing the quantity of oxygen in blood were corrected for dissolved oxygen. In the case of toadfish and goosefish the solubility coefficient for oxygen was experimentally established as 2.70 vols. per cent for 760 mm. O_2 at 25° C. This figure was assumed to hold in the case of the tautog.

In studies of the effect of acid on the ability of blood to combine with oxygen, samples of blood were equilibrated in oxygen or air to which had been added various amounts of carbon dioxide. When acidities were desired greater than could be obtained in this way a few drops of 4 per cent lactic acid were added.

The pH of fish blood is not easy to control and great difficulties were experienced in trying to establish dissociation curves at constant pH. On account of the great change in acidity of the hemoglobin with degree of oxygenation the use of a constant carbon dioxide tension does not lead to a constant pH at all oxygen tensions. This difficulty was overcome by grading the amount of carbon dioxide added and, in some cases, by adding a small amount of sodium bicarbonate. In this way it was possible to obtain points calculated to have approximately the same pH values at all degrees of oxygenation. The pH of the blood was calculated from gasometric data, using the Henderson-Hasselbalch equation and assuming pK 6.22 for whole blood at 25° C. Dissolved carbon dioxide was calculated by using Bohr's solubility coefficient slightly modified in a manner prescribed by Peters, Bulger, and Eisenman (1924) to allow for differences in corpuscular volume. The final factors used to calculate the volume percentage of dissolved CO_2 were 0.102 pCO_2 for toadfish, and 0.1 pCO_2 for the other fish bloods. It must be remembered that the electrolyte equilibrium between cells and plasma is completely ignored and the pH is calculated from determinations of the carbon dioxide equilibrium of whole blood rather than that of either plasma or cells.

The Apparent Loss of Oxygen Capacity in Acid Solutions

Root (1931) found that the amount of oxygen combined with certain fish bloods at a pressure of oxygen equivalent to that of air depended upon the acidity of the blood. The addition of very small amounts of carbon dioxide (even 5 or 10 mm.) or of lactic acid, appreciably decreased the oxygen bound. This effect is reversible, for if the blood equilibrated under acid conditions is again made alkaline it regains its original combining capacity.

It seemed to us that this phenomenon might be merely a pronounced "Bohr" effect (Bohr, Hasselbalch, and Krogh, 1904) in which the acid altered the oxygen dissociation constant to such an extent that the oxygen tension of air was insufficient to completely saturate the hemoglobin. If higher oxygen tensions had been used, saturation might have been complete and the oxygen capacity undiminished. To test this possibility fish blood was equilibrated with oxygen containing the requisite quantity of carbon dioxide. Since the total pressure was about one atmosphere, the oxygen tension of these gas mixtures decreased with increasing carbon dioxide concentration. For this reason lactic acid was added on some of the more acid samples to enable a relatively high acidity to be reached without too greatly diluting the oxygen with carbon dioxide.

The results upon the blood of the goosefish, the tautog, and the toadfish in both air and oxygen at 25° C. are represented in Fig. 1.

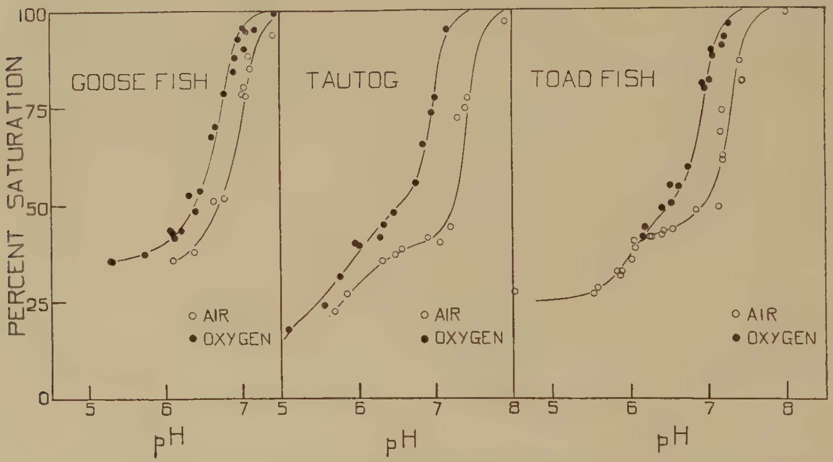

Fig. 1. The oxygen combined in fish bloods equilibrated with varying tensions of carbon dioxide and oxygen or of carbon dioxide and an oxygen tension approximating that of air.

The oxygen tension of the more acid points in the curves of the toadfish is about 500 mm., of the tautog 650 mm., and of the goosefish 350 mm. Except in the acid ranges of the toadfish blood, the samples equilibrated with oxygen combined with more oxygen than those treated with air. But it still remains a question as to whether the oxygen dissociation curves have shifted or whether the amount of oxygen which can be combined has been decreased. It seemed desirable, consequently, to determine the entire oxygen dissociation curves at various hydrogen ion concentrations in the hope that their analysis would enable us to determine the relative part played by these two phenomena.

Equations for Oxygen-Hemoglobin Equilibrium at Constant pH

There have been three types of equations describing oxygen dissociation which have proved useful for our purpose. The first of these is that of Hill (1910). The fraction of hemoglobin combined with oxygen, in the following manner:

$$Y = \frac{Kx^n}{1 + Kx^n}, \tag{1}$$

where x is the oxygen concentration expressed as pressure in mm. Hg. Equation (1) may be derived from the mass law on the assumption that n is the number of molecules of oxygen uniting with each molecule of hemoglobin.

According to Adair's hypothesis, each molecule of hemoglobin combines with four molecules of oxygen and the molecule $Hb(O_2)_4$ is built up and broken down in stages, thus:

$$Y = \frac{0.25K_1(O_2) + 0.5K_2(O_2)^2 + 0.75K_3(O_2)^3 + K_4(O_2)^4}{1 + K_1(O_2) + K_2(O_2)^2 + K_3(O_2)^3 + K_4(O_2)^4}, \tag{2}$$

in which K_1, K_2, K_3, and K_4 are products of mass law equilibrium constants. This equation has been successfully used to describe the curves for man (Adair, 1925), the horse (Ferry and Green, 1929), and the sheep (Ferry and Pappenheimer, 1929).

Another expression has been suggested by Redfield (Ferry and Green, 1929, p. 194) and has been found useful in describing the oxygen dissociation curves of a number of bloods containing hemocyanin which have a distinct undulatory character (Redfield, 1933). It may be assumed that the respiratory protein consists of two or more components, each of which reacts with oxygen independently of the others and reaches an equilibrium described by a distinct oxygen dissociation curve. Further, each component reacts with oxygen in accordance with Hill's equation (equation 1) but the components are each characterized by a different value of n. If the oxygen dissociation constants of the forms characterized by values of n of 1.0, 2.0, 3.0, 4.0, etc. are designated by K_1, K_2, K_3, K_4, etc., and the fraction of the total oxygen bound by each of these forms as α_1, α_2, α_3, α_4, etc., the fraction of the total respiratory protein present in the oxygenated condition, Y, is given by the equations:

$$Y = \frac{\alpha_1 K_1 x^1}{1 + K_1 x^1} + \frac{\alpha_2 K_2 x^2}{1 + K_2 x^2} + \frac{\alpha_3 K_3 x^3}{1 + K_3 x^3} + \frac{\alpha_4 K_4 x^4}{1 + K_4 x^4} \cdots$$

In applying this expression to the fish bloods which we have studied, it is not necessary to employ more than the first two terms so that the expression becomes

$$Y = \alpha_1 \frac{K_1(O_2)}{1 + K_1(O_2)} + \alpha_2 \frac{K_2(O_2)^2}{1 + K_2(O_2)^2}. \tag{3}$$

TABLE I

Oxygen Dissociation of Toadfish Blood Equilibrated at 25° C.

Experiment *	O_2 Pressure	O_2 Content	O_2 Combined	O_2 Capacity	O_2 Saturation	CO_2 Pressure	CO_2 Content	pH
	mm. Hg.	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*mm. Hg.*	*vol. per cent*	
17	8.76	1.35	1.32	6.98	18.9	38.8	67.3	7.42
	19.4	2.57	2.50		35.8	36.3	64.7	7.44
	25.3	2.96	2.87		41.1	34.7	65.8	7.47
	44.25	3.71	3.55		50.9	36.7	67.0	7.45
	69.8	4.47	4.22		60.5	34.3	62.9	7.45
	80.0	4.62	4.34		62.2	36.1	65.1	7.44
	90.9	5.11	4.79		68.6	34.0	63.0	7.45
	107.0	5.26	4.88		70.0	35.1	64.2	7.45
	115.0	5.28	4.87		69.8	36.4	63.2	7.43
	117.5	5.30	4.88		69.9	34.9	64.0	7.45
	134.2	5.81	5.33		76.4	34.75	63.2	7.45
	151.0	6.21	5.68		81.4	33.85	61.55	7.44
	172.0	6.56	5.95		85.3	36.3	61.4	7.41
	191.0	6.72	6.04		86.6	34.1	63.5	7.44
	261.5	7.27	6.34		90.8	35.4	58.2	7.40
19 †	2.69	0.47	0.46	7.21	6.39	46.3	48.2	7.18
	14.7	1.95	1.90		26.3	45.2	47.5	7.19
	26.9	2.81	2.72		37.7	42.5	47.4	7.22
	53.8	3.41	3.22		44.7	41.4	46.35	7.22
	80.0	3.77	3.49		48.4	41.3	46.85	7.22
	102.0	4.16	3.80		52.7	42.0	46.0	7.21
	131.4	4.54	4.07		56.5	41.0	46.2	7.21
	147.0	4.94	4.42		61.3	43.0	45.1	7.19
	163.4	5.24	4.66		65.2	38.9	44.7	7.23
	209.5	5.79	5.05		70.0	43.8	44.1	7.17
	247.0	6.31	5.44		75.5	40.75	42.8	7.19
	322.0	6.73	5.59		78.2	40.25	43.0	7.20
	410.0	7.44	5.99		83.8	39.5	41.5	7.19
	512.0	8.01	6.19		86.5	39.8	41.8	7.19
	723.0	9.10	6.53		90.6	39.2	41.2	7.19
18	4.23	0.71	0.69	7.15	9.70	35.6	23.6	6.96
	16.7	1.97	1.91		26.7	35.2	23.3	6.96
	25.1	2.53	2.44		34.1	34.9	22.5	6.95
	53.2	3.50	3.31		45.3	34.2	22.6	6.96
	107.1	3.89	3.51		49.2	34.4	23.05	6.96
	137.7	3.98	3.49		48.8	45.7	24.7	6.83
	150.0	4.11	3.58		50.0	32.0	22.2	6.98
	225.0	4.66	3.86		53.0	31.6	22.2	6.99
	291.0	5.18	4.15		58.0	34.2	24.8	7.01
	352.0	5.59	4.34		61.0	34.2	22.3	6.95
	388.0	6.06	4.68		65.5	33.6	23.6	6.99
	461.0	6.54	4.90		68.6	34.4	23.5	6.98
	500.0	6.93	5.16		72.1	33.3	21.4	6.94
	729.0	8.37	5.68		79.5	34.6	22.4	6.96

TABLE I—*Continued*

Experiment *	O_2 Pressure	O_2 Content	O_2 Combined	O_2 Capacity	O_2 Saturation	CO_2 Pressure	CO_2 Content	pH
	mm. Hg.	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*mm. Hg.*	*vol. per cent*	
6	4.23	0.65	0.64	6.04	10.6	51.85	28.3	6.86
	6.96	0.86	0.84		13.9	47.75	27.2	6.88
	16.65	1.70	1.65		27.2	50.7	27.2	6.85
	33.9	2.30	2.19		36.2	59.6	29.0	6.80
	69.9	2.76	2.53		41.8	53.9	26.9	6.81
	96.6	3.05	2.73		45.2	53.9	32.5	6.92
9	200.	3.62	2.91	6.20	47.0	46.3	26.2	6.89
	285.	3.98	2.97		48.0	47.7	30.3	6.94
	331.	4.30	3.13		50.5	46.6	25.9	6.88
	345.	4.60	3.38		54.5	47.3	25.2	6.86
	402.	5.02	3.60		58.0	44.5	22.7	6.83
	481.	5.32	3.61		58.2	46.6	25.8	6.87
	580.	5.59	3.54		57.1	45.5	26.9	6.91
	649.	5.94	3.64		58.7	46.1	24.5	6.86
5	4.90	0.61	0.61	6.16	9.9	120.2	39.55	6.57
	8.17	0.89	0.88		14.3	119.4	39.65	6.57
	12.74	1.18	1.16		18.8	110.8	38.25	6.60
	20.82	1.65	1.57		25.5	129.5	40.55	6.54
	36.14	2.05	1.90		30.8	123.0	40.00	6.56
	69.2	2.56	2.33		37.8	125.5	41.05	6.56
14	6.05	0.665	0.64	6.06	10.7	100.7	18.68	6.13
	13.10	1.015	0.97		16.0	100.2	18.40	6.12
	23.45	1.38	1.30		21.5	100.5	18.33	6.12
	33.7	1.63	1.51		24.9	97.2	18.10	6.13
	40.8	1.90	1.76		29.0	98.1	17.80	6.11
	48.7	2.02	1.85		30.5	97.7	17.48	6.10
	58.6	2.21	2.00		33.0	98.8	17.95	6.11
	91.0	2.44	2.12		35.1	97.4	18.00	6.13
	140.6	2.84	2.34		38.6	105.3	18.17	6.06

* Each experiment number represents measurements made on a single collection of blood.

† Sodium bicarbonate added to the blood.

In order to take account of the apparent inability of all of the hemoglobin to combine with oxygen, which characterizes the more acid samples, it is necessary to introduce a term α_0, equal to the fraction of total hemoglobin incapable of combining with oxygen. It follows that

$$\alpha_0 + \alpha_1 + \alpha_2 = 1.$$

THE OXYGEN DISSOCIATION CURVES OF TOADFISH BLOOD

The results of the experiments on the blood of the toadfish are given in Table I and the oxygen dissociation curves at constant pH are shown in Fig. 2.

These curves may be observed to have certain interesting characteristics regardless of the theory or of the equations used to describe the oxygen-hemoglobin equilibrium. The first of these is a difference in the shape of the curves at different hydrogen ion concentrations. In mammalian blood, on the contrary, curves at constant carbon dioxide tension or at constant pH are apparently of one family having the same shape.

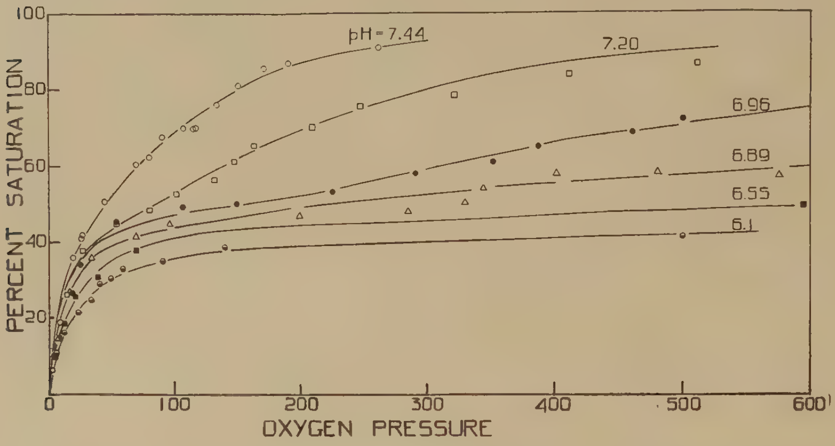

FIG. 2. Oxygen dissociation curves at constant pH of the blood of the toadfish. The curves have been drawn according to equation (3) using the following constants:

pH	α_1	α_2	α_0	K_1	$K_2 \times 10^4$	$1/K_1$	$1/K_2$
7.44	.5	.5	0	.11	.826	9	110^2
7.20	.5	.5	0	.10	.186	10	232^2
6.96	.5	.48	.02	.10	.033	10	548^2
6.89	.5	.2	.3	.071	.033	14	548^2
6.55	.5	0	.5	.0475		21	
6.1	.43	0	.57	.0475		21	

The data may be equally well described by equation (2) when the following values for the constants are used:

pH	K_1	$K_2 \times 10^2$	$K_3 \times 10^4$	$K_4 \times 10^6$
7.44	.2	1.0	.01	1.0
7.20	.1	.6	.04	.10
6.96	.1	.5	.001	.015
6.89	.1	.4	.001	.004
6.55	.1	.2	.001	.0001

A second effect of pH on these curves is a shift to the right with increasing acidity, a phenomenon well established for various mammalian bloods. On the other hand, the change in the position of the curve with change in pH is almost insignificant in the blood of the elasmobranch, *Raia ocellata* (Dill, Edwards, and Florkin, 1932). In these marine teleosts, and especially in the toadfish, the effect is very marked. As a result the curves determined at pH 7.2, 6.96, and 6.89 are not completely saturated even in the presence of 500 mm. O_2 and the shape of the curves indicates that more oxygen would be combined at higher oxygen tensions.

This is an explanation of a part of the decrease in oxygen capacity described by Root (1931). It should be noted, however, that the curves determined at pH 6.55 and 6.1 are practically flat at tensions of oxygen above 150 mm. which suggests that saturation is complete but that a part of the hemoglobin is incapable of combining with oxygen.

The next important feature of the curves is their peculiar shape. Some of them are not simple hyperbolic or sigmoid curves but are undulatory in character, having two regions concave to the abscissa. Similar types of curves have been found for the blood of some birds (Wastl and Leiner, 1931) and of certain animals containing hemocyanin (Redfield, 1933). The break between the two regions of the curves occurs at about 50 per cent saturation, and the more acid curves, in which the combined oxygen has become constant, do not rise above 50 per cent saturation. This characteristic of the curves suggests that the oxygenation of the hemoglobin consists of two steps, or that there are two components to the curves representing the behavior of different fractions of the pigment present in equal quantities. Theoretical treatment of these possibilities is provided by the equations given above.

The equation (1) developed by Hill applies only to simple curves, hyperbolic or sigmoid in shape, and thus could be used to describe only the most acid curves and these only if the decrease in oxygen capacity is assumed to be real so that higher oxygen tensions would not increase the oxygen bound.

Although Adair's theory in which the hemoglobin combines with oxygen in steps was derived for sigmoid curves, equation (2) may be used to describe the oxygen dissociation curves of the toadfish. By making K_3 and K_4 sufficiently small, the terms in which they appear become ineffective at the oxygen tensions used and the curve becomes flat at one half saturation, as in the experiment at pH 6.55. According to this treatment the decrease in oxygen capacity is only apparent and the effect really one on the magnitude of the constants and thus a "Bohr" effect. However, the curve at pH 6.1 is indubitably flat over a long range at less than 50 per cent saturation, that is, at 43 per cent, and it is also apparent from Fig. 1 that a further increase in acidity would further decrease the oxygen bound. Such curves can only be derived from the Adair equation by assuming a true loss in oxygen capacity, thus necessitating the introduction of a new constant to describe this loss.

The curves may be described equally well in terms of Redfield's equation which employs fewer constants. The smooth curves in Fig. 2 are drawn according to equation (3). When the curves have not flattened out at one atmosphere of oxygen, the fraction of hemoglobin incapable of combining with oxygen must be calculated from the shape of the dissociation curves at lower tensions and this unfortunately gives an additional degree of freedom in analyzing some of the curves.

The difference between the values for K_1 and K_2 gives the undulatory character to the curves. In some cases the hemoglobin combining with oxygen according to the first constant is almost completely saturated before the second inflection begins. By taking α_1 and α_2 each $= 0.50$, which implies that the amount of hemoglobin acting as though it combined with one molecule of oxygen at a time exactly equals that combining with two molecules, the flat places in the curves or the points where there is a change of inflection occur at about one half saturation.

The decrease in oxygen capacity takes place first at the expense of the component behaving as though $n = 2$. The value of α_1, the amount of hemoglobin behaving according to the first term of the equation, remains constant at 0.50 throughout the pH range until the amount of hemoglobin capable of combining with oxygen has been reduced to 50 per cent of the whole. Thus, the curve at pH 6.55, in which the capacity is 50 per cent of the total oxygen-combining power, is a rectangular hyperbola. Further addition of acid reduces the capacity but the curve is still of the same shape and has the same value for the constant K_1. This implies that the effect described by Root comprises a real inactivation of the hemoglobin and is due only in part to a greatly exaggerated "Bohr" effect.

All three treatments have certain common implications. The description of the curves in accordance with either equation (1), (2), or (3) requires the assumption that a portion of the hemoglobin has lost its ability to combine with oxygen in the case of the most acid solution. In the use of equation (3) it is implied that the prosthetic groups responsible for the two components of the curves are divided in equal amounts. This condition could be met if there were two forms of hemoglobin present, but the probability of two independent substances existing in such exact proportion is rather small. It is much simpler to postulate the existence of a number of oxygen-combining groups on one molecule, one half of which behave in one way and one half in the other. Since one half of the group behave as though they combined with oxygen in pairs, at least four prosthetic groups must be attributed to the molecule. In this regard the implications regarding the structure of the hemoglobin molecule are the same as those postulated by Adair in deriving equation (2).

THE OXYGEN DISSOCIATION CURVES OF THE BLOOD OF THE TAUTOG AND OF THE GOOSEFISH

The blood of the tautog and of the goosefish may be considered together since they exhibit similar characteristics. The data are presented in Tables II and III.

The most important characteristic of these curves is a change in the shape of the curve with a change in pH. Equation (1) may be transformed to the logarithmic form:

$$\text{Log}\,\frac{\text{HbO}_2}{\text{Hb}} = \log K + n \log \text{pO}_2.$$

When log HbO_2/Hb is plotted against log pO_2, the resulting curve is a straight line, the slope of which is equal to n. The data for these two fish bloods have been calculated in this manner and the results plotted in Fig. 3. In both cases the most alkaline curves are straight lines and the slope is 2.0, that is, Hill's equation is applicable and n is 2.0. In both cases, also, the most acid curves are straight lines but here n is 1.0 and consequently the oxygen dissociation curves are rectangular hyperbolæ.

In both these bloods the oxygen-combining power is decreased in the more acid solutions. In the tautog the curve has changed to a rectangular hyperbola ($n = 1.0$) at pH 7.2. At this reaction the oxygen capacity is not appreciably lowered, so that the change in shape is independent of this phenomenon. In the goosefish the decrease in oxygen capacity begins to appear before the curves have assumed a shape characterized by $n = 1$, and in applying Hill's equation it is necessary to make allowances for the decreased oxygen capacity of the blood, as was done in the case of toadfish blood (p. 391). At pH 6.84, 6.80, and 6.1 the oxygen capacities are assumed to be 88, 80, and 54 per cent respectively, of those obtaining in alkaline solutions.

TABLE II

Oxygen Dissociation of Goosefish Blood Equilibrated at 25° C.

Experiment *	O_2 Pressure	O_2 Content	O_2 Combined	O_2 Capacity	O_2 Saturation	CO_2 Pressure	CO_2 Content	pH
	mm. Hg.	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*mm. Hg.*	*vol. per cent*	
3	2.77	2.80	2.79	8.40	33.2	2.53	24.1	8.20
	7.08	5.70	5.68		67.5	3.16	25.2	8.11
	10.4	7.02	6.98		83.0	2.14	22.9	8.23
	15.94	7.90	7.84		93.3	2.41	23.2	8.20
	66.9	8.74	8.50		101.0	2.76	24.2	8.15
4 †	5.26	5.07	5.05	8.59	58.8	6.36	51.3	8.12
	6.79	5.64	5.62		65.5	8.54	56.2	8.03
	7.74	6.42	6.39		74.4	6.17	50.4	8.13
	7.89	6.35	6.32		73.6	7.23	54.3	8.09
	12.33	7.50	7.46		86.8	7.20	53.9	8.09
	14.58	7.96	7.91		92.2	6.29	52.0	8.15
	25.1	8.32	8.23		95.8	6.22	51.2	8.13
3	3.19	0.93	0.93	8.40	11.1	0.98	2.79	7.65
	21.3	5.12	5.04		60.0	0.91	2.8	7.52
	25.8	5.82	5.73		68.2	0.93	2.07	7.55
	32.3	6.36	6.25		74.3	0.61	1.6	7.62
	58.0	7.54	7.33		87.4	1.10	2.3	7.52
4 †	9.86	1.22	1.18	8.59	13.7	48.3	39.5	7.08
	25.7	3.09	3.00		35.0	45.6	37.5	7.08
	31.35	3.67	3.56		41.4	47.2	37.3	7.06
	39.85	4.45	4.31		50.3	43.5	35.0	7.07
	68.7	5.92	5.68		66.2	42.3	33.0	7.07
	109.6	7.36	6.97		81.0	36.4	30.1	7.08
	151.	8.11	7.58		88.3	33.2	28.4	7.10
	182.	8.65	8.00		93.3	33.8	28.3	7.09
4 †	12.0	1.41	1.37	8.59	15.9	49.6	39.6	7.06
	25.1	2.76	2.67		31.1	49.7	39.2	7.06
	37.4	3.45	3.32		38.6	50.7	37.8	7.03
	54.4	4.37	4.18		48.7	49.6	37.7	7.04
	104.6	7.00	6.63		77.3	42.4	32.8	7.05
	149.5	7.22	6.69		77.9	41.3	32.6	7.06
	148.2	7.43	6.90		80.3	41.1	31.5	7.04
	228.	8.38	7.57		88.3	41.2	30.3	7.03
3	4.05	0.55	0.54	8.40	6.45	50.7	26.0	6.84
	19.8	1.52	1.45		17.3	50.0	25.7	6.84
	27.35	2.15	2.05		24.4	50.7	26.2	6.84
	59.9	3.57	3.36		40.0	50.8	24.9	6.81
	102.6	4.31	3.95		47.2	50.3	24.9	6.82
	197.5	5.99	5.29		62.8	49.6	23.3	6.80
	303.5	6.92	5.84	8.28	70.5	46.7	22.0	6.79
	593.	8.73	6.62	8.19	80.7	46.4	20.7	6.76
	732.	9.05	6.45	7.94	81.3	48.5	21.3	6.75

TABLE II—*Continued*

Experiment *	O_2 Pressure	O_2 Content	O_2 Combined	O_2 Capacity	O_2 Saturation	CO_2 Pressure	CO_2 Content	pH
	mm. Hg.	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*mm. Hg.*	*vol. per cent*	
4	7.0	0.72	0.70	8.59	8.15	41.0	19.8	6.80
	12.8	1.12	1.07		12.5	41.8	20.2	6.80
	34.5	2.22	2.10		24.5	39.4	19.5	6.82
	80.4	3.87	3.58		41.7	38.8	16.6	6.74
	106.5	4.39	4.02		46.8	37.6	18.1	6.80
	150.	5.01	4.48		52.1	34.9	16.6	6.78
	250.	6.34	5.45		63.5	31.2	14.1	6.77
	743.	9.08	6.44		75.0	25.1	11.6	6.78
3	34.6	1.88	1.76	8.40	20.9	390.	70.6	6.13
	64.3	2.24	2.00	8.32	23.8	374.	67.2	6.12
	106.	2.88	2.50	8.03	31.2	404.	72.0	6.11
	150.	3.39	2.86	7.96	35.8	374.	66.6	6.11
	378.	4.35	3.01	7.24	41.6	377.	68.7	6.13

* Each experiment number represents measurements made on a single collection of blood.

† Sodium bicarbonate added to the blood.

The curves at intermediate pH values are such that *n* lies between 1 and 2. The curve for the tautog at pH 7.4 is apparently a straight line of slope 1.3 so that equation (1) is applicable whereas the intermediate goosefish curves would seem to be slightly curved, indicating that a more complicated equation would give a more satisfactory description.

There is no question but that equation (2) could be used to describe these curves but their simple character renders the application of such a complicated expression of little interest.

Equation (3) may also be successfully applied here. The smooth curves in Figs. 4 and 5 are drawn according to this equation using the constants given in the legends.

Thus the dissociation curves of the tautog and the goosefish are so simple in character that they may be described by equations based on any one of the theories of the mode of combination between oxygen and hemoglobin, and in themselves give no basis for a preference of one theory over another.

These curves leave little doubt that the decrease in oxygen in acid solution is really due to a loss in ability to combine with oxygen, and not to a "Bohr effect," for certain of the curves have the same dissociation constant and differ only by the total amount of oxygen with which they are able to combine when the acidity is changed. The

TABLE III

Oxygen Dissociation of Tautog Blood Equilibrated at 25° C.

Experiment *	O_2 Pressure	O_2 Content	O_2 Combined	O_2 Capacity	O_2 Saturation	CO_2 Pressure	CO_2 Content	pH
	mm. Hg.	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*mm. Hg.*	*vol. per cent*	
6 †	9.56	1.45	1.42	9.66	14.7	12.60	56.1	7.86
	13.13	3.04	2.99		30.9	12.5	55.0	7.85
	14.23	3.07	3.02		31.3	11.80	55.1	7.88
	21.8	4.90	4.82		50.0	10.97	52.4	7.89
	38.7	7.07	6.93		71.7	10.02	50.2	7.91
	42.9	7.73	7.58		78.5	10.35	50.1	7.90
	84.2	9.09	8.79		90.7	9.59	49.1	7.92
2	12.9	1.17	1.12	8.74	12.7	9.13	15.05	7.41
	15.2	1.52	1.47		16.7	7.62	13.50	7.45
	22.6	1.97	1.89		21.5	7.89	14.35	7.46
	46.7	4.24	4.06		46.1	8.08	12.33	7.38
	70.1	5.10	4.85		55.0	7.98	12.12	7.38
	155.	7.33	6.80		77.2	5.73	9.50	7.41
	276.	8.92	7.95		90.2	5.11	8.06	7.40
4	16.4	1.38	1.32	9.39	14.2	25.8	26.1	7.18
	52.5	2.89	2.71		29.1	26.3	26.3	7.17
	109.5	4.89	4.50		47.9	19.86	22.3	7.23
	152.5	5.62	5.08		54.1	21.1	22.2	7.20
	214.5	6.45	5.69		61.1	18.8	20.8	7.22
	268.	7.08	6.13		65.9	19.3	20.5	7.20
	381.	8.37	7.02		75.5	16.8	17.8	7.20
3	13.9	1.10	1.05	7.66	13.7	118.8	36.8	6.54
	30.5	1.78	1.67		21.8	116.6	36.2	6.54
	53.8	2.30	2.11		27.5	118.5	36.5	6.54
	87.9	2.59	2.28		29.7	137.4	38.9	6.48
	124.7	3.14	2.70		35.3	130.2	36.6	6.48
	147.0	3.46	2.94		38.4	106.8	33.6	6.55
	150.8	3.36	2.83		37.0	130.7	36.8	6.48
	163.8	3.61	3.03		39.5	117.6	36.5	6.54
	309.0	4.62	3.52		45.8	123.0	36.4	6.51
	625.0	5.91	3.69		48.0	134.2	37.6	6.46
5 ‡	23.3	0.82	0.74	9.54	7.74	107.3	14.33	5.73
	39.6	1.34	1.20	8.38	14.3	104.5	15.10	5.87
	54.2	1.39	1.20	7.87	15.2	106.0	13.60	5.67
	81.9	1.55	1.26	7.77	16.2	110.8	14.49	5.71
	101.8	2.25	1.89	8.44	22.4	107.0	14.94	5.82
	155.0	2.33	1.78	8.00	22.31	109.8	14.18	5.69
	670.0	5.06	2.68	8.52	31.4	108.2	14.46	5.76

* Each experiment number represents measurements made on a single collection of blood.

† Sodium bicarbonate added to the blood.

‡ Lactic acid added to the blood.

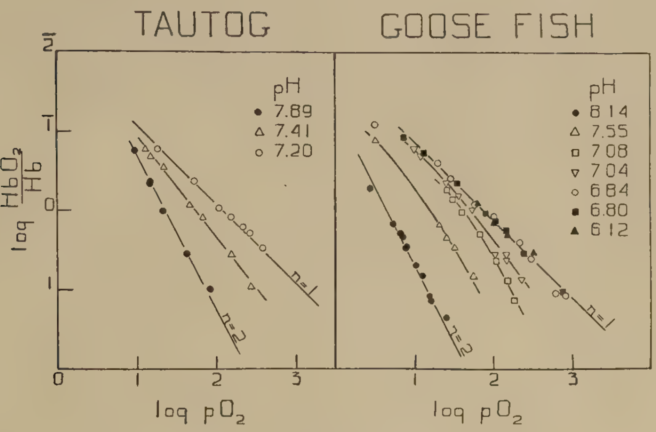

FIG. 3. For explanation of this figure, see text.

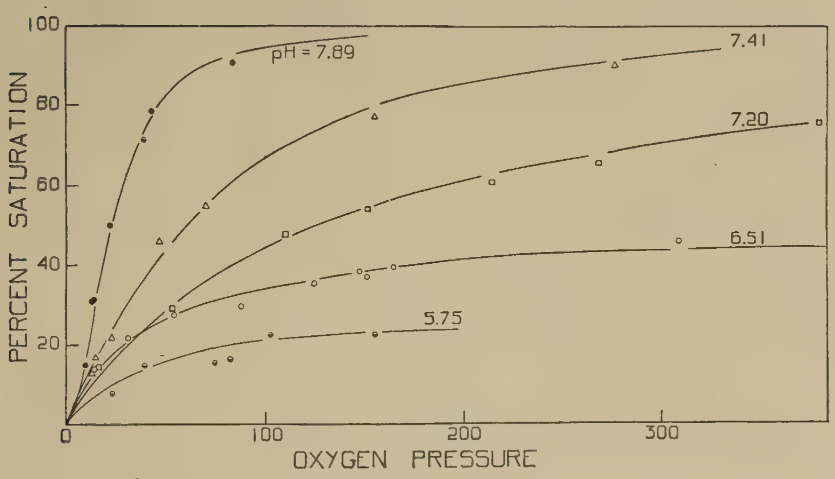

FIG. 4. Oxygen dissociation curves at constant pH of the blood of the tautog. The curves have been drawn according to equation (3) using the following constants:

pH	α_1	α_2	α_0	K_1	$K_2 \times 10^2$	$1/K_1$	$1/K_2$
7.89	0	1.0	0		.19		23^2
7.41	.5	.5	0	.0286	.0156	35	80^2
7.20	1.0	0	0	.008		125	
6.51	.5	0	.5	.022		45	
5.75	.3	0	.7	.022		45	

more acid curves for tautog blood are rectangular hyperbolæ and K_1 is the same at pH 6.5 and 6.1 although the oxygen capacity is

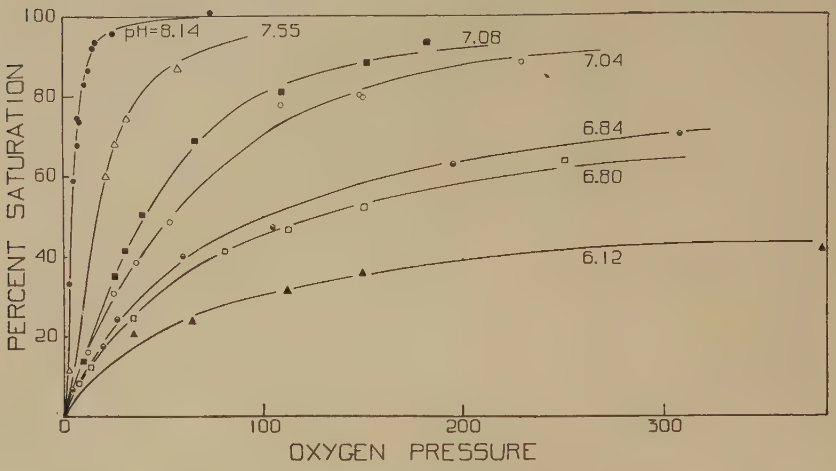

FIG. 5. Oxygen dissociation curves of the blood of the goosefish at constant pH. The curves have been drawn according to equation (3) using the following constants:

pH	α_1	α_2	α_0	K_1	K_2	$1/K_1$	$1/K_2$
8.14	0	1.0	0		.05		4.47^2
7.55	.25	.75	0	.067	.0011	15	30^2
7.07	.45	.55	0	.04	.0004	25	50^2
7.04	.50	.50	0	.04	.000158	25	80^2
6.84	.88	0	.12	.0131		76	
6.80	.80	0	.20	.0131		76	
6.12	.54	0	.46	.0131		76	

diminished. The same is true of the goosefish blood at pH 6.84, 6.80, and 6.12.

The change in the position of the curves with pH is very marked in these bloods as well as in that of the toadfish. In the tautog there is an additional phenomenon. The oxygen dissociation constant for the curve at pH 7.2 is considerably smaller than that for the curves at lower pH values. That is, at first the curves are shifted to the right with increasing acidity but at higher acid concentrations there is a reversal of the effect. A similar reversal of the Bohr effect was found by Rona and Ylppö (1916) working on dog blood, by Ferry and Green (1929) for horse hemoglobin, and by Stedman and Stedman (1926) and Hogben (1926) for crustacean hemocyanin.

The most interesting single characteristic of these curves is the change in shape from a rectangular hyperbola to a sigmoid curve. Unlike the toadfish, in which one component behaves as though $n = 2$ and one component as though $n = 1$ at all pH values, the change in shape must be due to a change in behavior of the component groups.

Thus, whatever the theory used as a basis for the description of the curves, it may be observed that acidity has a threefold effect on the blood; first, a change in the value of the oxygen dissociation constant, second, a decrease in oxygen capacity, third, a change in the shape of the curves describing the equilibrium with oxygen.

THEORETICAL DEDUCTIONS CONCERNING THE LOSS IN OXYGEN CAPACITY IN ACID SOLUTIONS

Henderson (1920) has shown that the effect of acid on the combining power of hemoglobin with oxygen could be described in terms of four mass law expressions; one for the combining power of oxygen with acid hemoglobin, HHb, one for oxygen with salt hemoglobin, BHb, and one each for the acid dissociation of oxygenated and reduced hemoglobin, $HHbO_2$ and HHb respectively. Thus the ease with which oxygen combines with hemoglobin depends upon the dissociation of the latter as an acid and oxyhemoglobin is a stronger acid than reduced hemoglobin.

The situation becomes rather complicated when more than one molecule of oxygen is considered to combine with one molecule of hemoglobin. By making certain assumptions concerning the interdependence of the four prosthetic groups, it is possible to derive an expression predicting the relation between oxygen capacity and hydrogen ion concentration exhibited by the data in Fig. 1. By making a few assumptions it is possible to explain this phenomenon of reversible loss of oxygen capacity in relatively simple chemical terms. The fundamental conception is that the oxygen combination is intimately dependent upon the acid dissociation of the hemoglobin as in mammalian blood, but that the effect is so exaggerated that oxygen, at ordinary oxygen tensions, will not combine with the hemoglobin unless the latter is in the ionized state. This is, of course, not the only possible theory but it is set forth here as a simple interpretation of the mechanism of a phenomenon apparently very different from our usual conception of the equilibrium between oxygen and hemoglobin.

Let it be assumed:

1. That each hemoglobin molecule contains four groups capable of

combining with oxygen and that each of these groups is associated with the dissociation of two hydrogen ions.[2]

2. Assume further that only the ionized prosthetic groups, that is, the groups from which hydrogen ions have been dissociated, can combine with oxygen.[3] Then in the presence of oxygen sufficient to combine with all the ionized hemoglobin the number of forms present is reduced to H_8Hb, $H_6HbO_2^{=}$, $H_4Hb(O_2)_2^{\equiv}$, $H_2Hb(O_2)_3^{\equiv=}$, and $Hb(O_2)_4^{\equiv\equiv}$.

3. Assume finally that the first two pairs of H ions dissociate from the hemoglobin separately but the last two pairs dissociate in a single step; then there never is present an appreciable amount of the form which can combine with three molecules of oxygen. This is in accordance with the usage of equation (3) as applied to the oxygen dissociation curves of toadfish blood.

The equilibrium reactions for the acid and oxygen dissociation of the hemoglobin molecule according to these restrictions are reduced to

$$H_8Hb = H_6Hb^{=} + 2H^+ \qquad H_6Hb^{=} + O_2 = H_6Hb(O_2)^{=}$$

$$H_6Hb(O_2)^{=} = H_4HbO_2^{==} + 2H^+ \qquad H_4HbO_2^{==} + O_2 = H_4Hb(O_2)_2^{==}$$

$$H_4Hb(O_2)_2^{==} = Hb(O_2)_2^{\equiv\equiv} + 4H^+ \qquad Hb(O_2)_2^{\equiv\equiv} + 2O_2 = Hb(O_2)_4^{\equiv\equiv}$$

If the oxygen tension is sufficiently high to convert practically all of the hemoglobin from which the hydrogen has been dissociated into the corresponding oxygenated form, the intermediate product of each pair of equations is never present in appreciable amount and the following equilibrium reactions and mass law expressions may be assumed to describe the limiting conditions:

$$H_8Hb = H_6HbO_2^{=} + 2H^+ \qquad \frac{[H_6HbO_2^{=}]}{[H_8Hb]} = \frac{K_1}{[H^+]^2}$$

$$H_6HbO_2^{=} = H_4Hb(O_2)_2^{=} + 2H^+ \qquad \frac{[H_4Hb(O_2)_2^{==}]}{[H_6HbO_2^{=}]} = \frac{K_2}{[H^+]^2}$$

$$H_4Hb(O_2)_2^{\equiv} = Hb(O_2)_4^{\equiv\equiv} + 4H^+ \qquad \frac{[Hb(O_2)_4^{\equiv\equiv}]}{[H_4Hb(O_2)_2^{==}]} = \frac{K_3K_4}{[H^+]^4}$$

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[2] The assumption that the combination of each oxygen molecule is accompanied by the dissociation of two hydrogen ions is in accordance with the finding of Ferry and co-workers (1929) that K_1 in equation (2), as applied to horse hemoglobin and to sheep blood, varies with the square of the hydrogen ion concentration, and with the observation of Redfield and Ingalls (1932) that the oxygen dissociation constant for certain hemocyanins also varies with the square of the hydrogen ion concentration.

[3] This also has a parallel in the hemocyanins, where the addition of acid produces a colorless compound incapable of combining with oxygen. See the end of this discussion.

The relative concentrations of the different forms present at any given pH may be readily calculated either by the logarithmic form usually employed in treating titration curves or in an algebraic form similar to the oxygen dissociation equations. Thus for the first equilibrium if

$$[H_8Hb] + [H_6HbO_2^{=}] = 1,$$

then

$$\frac{[H_6HbO_2^{=}]}{1 - [H_6HbO_2^{=}]} = \frac{K_1}{[H^+]^2} \text{ and } [H_6HbO_2^{=}] = \frac{K_1}{[H^+]^2 + K_1}$$

and similarly for the other expressions,

$$[H_4Hb(O_2)_2^{\equiv}] = \frac{K_2}{[H^+]^2 + K_2}, \text{ and } [Hb(O_2)_4^{\equiv\equiv}] = \frac{K_3K_4}{[H^+]^4 + K_3K_4}.$$

The total amount of oxygen bound at any given pH is the sum of all of the oxygenated forms.

$$H_6HbO_2^{=} + H_4Hb(O_2)_2^{\equiv} + Hb(O_2)_4^{\equiv\equiv}$$

Since only one quarter of the total oxygen bound can be in the form $H_6HbO_2^{=}$, the concentration as derived from the equilibrium expression must be multiplied by 0.25 as must also the concentration of $H_4Hb(O_2)_2^{\equiv}$, while the concentration of $Hb(O_2)_4^{\equiv\equiv}$ must be multiplied by 0.50. Substituting these values and the mass law expressions for the various oxygenated forms the total oxygen combined, Z, becomes

$$Z = .25\frac{K_1}{[H^+]^2 + K_1} + .25\frac{K_2}{[H^+]^2 + K_2} + .50\frac{K_3K_4}{[H^+]^4 + K_3K_4}.$$

In applying these equations to the data in Fig. 1, it must be kept in mind that the shape of the oxygen dissociation curves indicates that the hemoglobin would combine with slightly more oxygen if the oxygen tension were sufficiently increased. The theoretical oxygen capacity may be calculated in the more acid range because the curves are simple rectangular hyperbolæ and in all three bloods the oxygen dissociation constant has the same value in the most acid curves. If it be assumed that the constant is the same at intervening and lower pH values, knowing the oxygen tension and the concentration of oxygenated hemoglobin, it is possible to calculate the limiting amount of oxygen with which the hemoglobin can combine at these reactions. These values have been calculated using the data given in Fig. 1 for the goosefish below pH 6.8 where $K_1 = .013$, for the tautog below pH. 6.55 where $K_1 = .022$, and for the toadfish below 6.5 where

$K_1 = .047$. At higher pH values the capacities have been calculated somewhat less accurately. The points between pH 6.55 and 6.96 for the tautog have been calculated using the K_1 values at both these reactions, then averaging the results which are then ± 2 per cent of the correct value. At oxygen tensions of 700 mm. the first half of the toadfish hemoglobin is practically saturated and the capacity between pH 6.55 and 7.0 has been calculated according to equation (3) using K_2 as 3.33×10^{-6}. All of the calculated points are represented in Fig. 6.

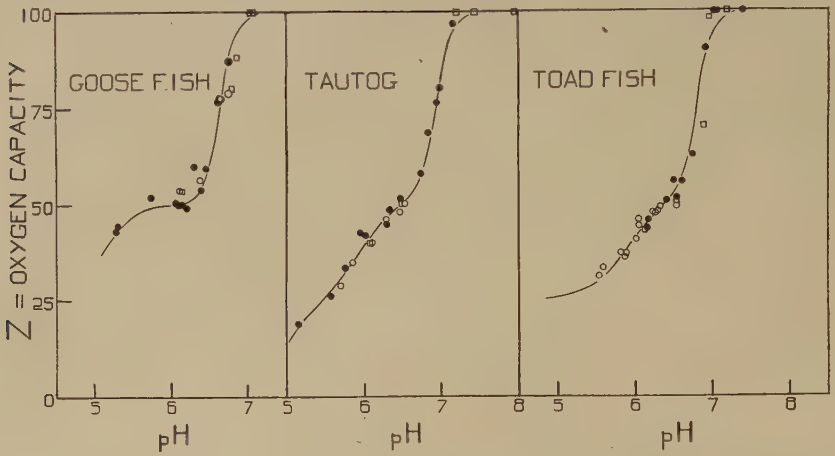

FIG. 6. The oxygen capacity of certain fish bloods. The points are calculated as described in the text:

● from the blood equilibrated with oxygen as given in Fig. 1.
○ from the blood equilibrated with air as given in Fig. 1.
☐ from the oxygen dissociation curves in Figs. 2, 4, and 5.

The curves are drawn according to equation (4).

The curves in this figure are drawn according to equation (4) where $\sqrt{K_2}$ and $\sqrt[4]{K_3K_4}$ are for the goosefish $10^{-5.1}$ and $10^{-6.65}$ respectively; for the tautog, $10^{-5.93}$ and $10^{-6.95}$ respectively, and for the toadfish $10^{-5.85}$ and $10^{-6.8}$ respectively. $\sqrt{K_1}$ is approximately 10^{-5} for the tautog but is too large to be determined for the other two fishes.

The applicability of this type of equation to the oxygen capacity supports the interpretation of the oxygen dissociation curve as developed in the case of the toadfish. The equation is also adequate to describe the relation observed in tautog and goosefish blood, although the assumptions used in deriving curves to describe the oxygen equilibrium are somewhat different from those employed for toadfish blood. This difference suggests that in some cases there may be an interdependence of the acid dissociation of the prosthetic groups which is not paralleled by an interdependence in the combination of oxygen.

The most important implication of the curves in Fig. 6 is the same as that derived from the undulatory character of the oxygen dissociation curves of the toadfish, namely, hemoglobin contains prosthetic groups differing in their behavior with respect to hydrogen dissociation and oxygen combination.

The evidence for this is the inflection of the tautog and toadfish curves at 50 per cent saturation and the actual break in the goosefish curve at the same point. Thus the effect of the addition of acid, as originally suggested (Root, 1931), may be interpreted as the inactivation of prosthetic groups on the hemoglobin molecule. This inactivation is probably due to the undissociated character of the hemoglobin as an acid, oxygen combining with only the ionized form of hemoglobin.

A similar inactivation of respiratory pigment in the presence of acid has been described by Redfield, Mason, and Ingalls (1932). The hemocyanin of *Limulus polyphemus* reacts with hydrochloric acid to form a component that is colorless and does not react with dissociable oxygen. The colorless component may be separated from a partially acidified hemocyanin solution by the addition of a strong solution of sodium chloride. When such a separation has been effected, analysis of the nitrogen in the filtrate indicates that its protein content has been diminished to just such an extent as the hemocyanin has been decolorized. Here, also, the combination of each oxygen molecule appears to be dependent upon the dissociation of two hydrogens, for the oxygen dissociation curves are rectangular hyperbolæ and the equilibrium between hydrochloric acid, hemocyanin, and the resulting colorless component may be described by an equation in which the hemocyanin is behaving as a divalent acid or base.

We wish to express our thanks to Professor Alfred C. Redfield for his generous interest and direction throughout the course of this investigation.

SUMMARY

1. The oxygen dissociation curves of the blood of certain marine teleosts, the toadfish, the goosefish, and the tautog, have been studied.

2. The oxygen dissociation curves for the toadfish are undulatory in character with two areas concave to the abscissa. The second inflection begins at approximately one half saturation.

3. The oxygen dissociation curves for the goosefish and the tautog change shape with change in pH. At alkaline reactions the curves are sigmoid whereas in acid solutions they are rectangular hyperbolæ.

4. The form of the oxygen dissociation curves at constant pH can be described by Hill's equation only under a limited number of circumstances. The curves for the toadfish may be described in terms of equations derived from the assumption that each molecule of hemoglobin combines with four molecules of oxygen.

5. The position of the dissociation curves of all of these fish bloods is markedly affected by pH, and, in addition, with increasing acidity the oxygen capacity is reduced.

6. The manner in which the addition of acid lowers the oxygen capacity may be deduced from the assumption that oxygenation is dependent upon the dissociation of hemoglobin as an acid.

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Contribution No. 8

Reprinted from Biological Bulletin, Vol. LXIV, No. 2, April, 1933

STUDIES ON THE BIOLOGY AND CHEMISTRY OF THE GULF OF MAINE

I. Chemistry of the Waters of the Gulf of Maine in August, 1932

NORRIS W. RAKESTRAW

The Woods Hole Oceanographic Institution[1]

The hydrographic features of the Gulf of Maine—temperature, salinity, and the dominant concentration of its waters—are now known broadly speaking (4) (5). The same may be said of the general occurrence of its principal inhabitants (3). The final goal of such studies, the explanation of the natural economy of these inhabitants in terms of their physical and chemical environment, lies ahead. It is to be sought not so much by the generalized surveys of the area as by more detailed study of the physical and, more especially, of the chemical changes in the water from season to season at selected locations, combined with simultaneous observations on the occurrence, abundance, and distribution of the animals and plants.

Oceanic biologists in various parts of the world have devoted much attention during the past few years to the factors and events that control the biologic fertility of the seas. Parallelism has so repeatedly been established between variations in the abundance of phytoplankton on the one hand and in the available supply of plant-nutrients on the other, that a causative relationship is now universally accepted. Much of detail is yet to be learned not only regarding the nutritive requirements of different groups of planktonic plants, but equally as to the situation existing with regard to the cycle of limiting substances in different parts of the sea.

The Gulf of Maine appears to be a particularly favorable area for such detailed studies. Within a circumscribed region of ready accessibility there occurs a variety of hydrographic conditions, in many ways representative of boreal seas as a whole. These range from the turbulent shallow waters overlying the offshore banks to the stable and stratified waters which develop in the deeper portions of the Gulf during the summer. Within this area exceptionally large ranges of temperature occur, both seasonal and in correlation with the depth of the water. Furthermore, the deeper portions of the Gulf appear to be fed by a tongue of water originating at even greater depths offshore. This water will be shown from its chemical content to be characteristic of the more stagnant waters of the deep sea. Conditions are consequently favorable for the examination of the nutrient cycle in all its phases.

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[1] Contribution No. 8, Woods Hole Oceanographic Institution.

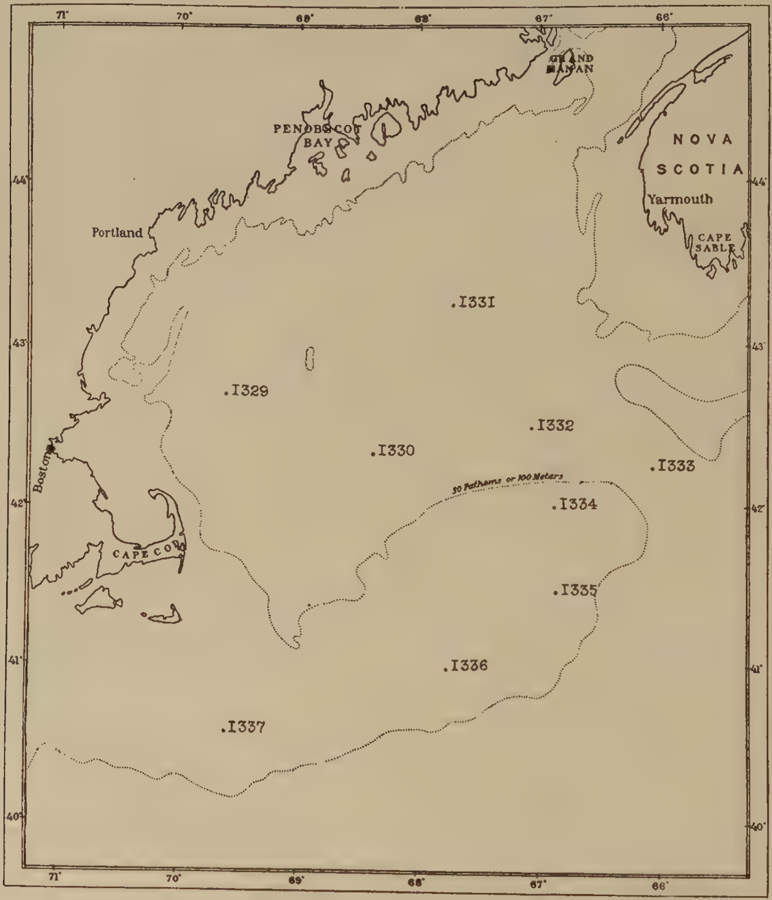
71°
70°
69°
68°
67°
66°
GRAND MANAN
PENOBSCOT BAY
NOVA SCOTIA
Yarmouth
CAPE SABLE
Portland
44°
43°
42°
41°
40°
.1331
.1329
.1332
.1330
.1333
50 Fathoms or 100 Meters
.1334
Boston
CAPE COD
.1335
.1336
.1337

Fig. 1. Locations of "Atlantis" Stations 1329–1337, August 2–5, 1932.

During the month of August, 1932, a cruise of the research ship "Atlantis" afforded an opportunity for the simultaneous study of the quantitative distribution of phytoplankton, of bacteria, and of the most obviously important nutrient substances of the sea water. Stations were made in two regions of striking contrast, the deep basin of the Gulf on the one hand, and the waters over its shoal southern rim (Georges Bank) on the other. The location of the stations is charted in Fig. 1 and their position recorded in Table I.

The present paper deals with the chemical observations made during this cruise. The results of the study of phytoplankton and bacteria are presented in the succeeding communications.

The chemical program of the cruise consisted in the determination of phosphate, nitrate, nitrite, and oxygen in the water at various depths from all stations. The methods used were those which have now become rather well standardized: for phosphate, the colorimetric molybdic acid method successfully used by Atkins and many others (1) (2) (3) (8); for nitrate, the colorimetric method of Harvey (7) (8), using reduced strychnine; for nitrite, the Griess-Ilosvay method, but substituting the diethyl derivative of alpha-naphthylamine; for oxygen, Winkler's iodimetric titration. All analyses were carried out on ship-board, as soon as possible after the water samples had been taken. The oxygen and phosphate analyses were made by Mr. T. S. Greenwood.

TABLE I

Location of Stations

Station No.	Date	Lat. N.	Long. W.
1329	August 2, 1932	42° 42′	69° 36′
1330	August 2, 1932	42° 19′	68° 22′
1331	August 3, 1932	43° 14′	67° 45′
1332	August 3, 1932	42° 28′	67° 03′
1333	August 4, 1932	42° 15′	66° 04′
1334	August 4, 1932	42° 00′	66° 54′
1335	August 4, 1932	41° 26′	66° 53′
1336	August 5, 1932	40° 57′	67° 49′
1337	August 5, 1932	40° 35′	68° 42′

From the chemical standpoint as well as the hydrographic the stations may be divided into two groups; the first five, Nos. 1329–1333, fairly deep, in the Gulf of Maine; and the last four, Nos. 1334–1337, much shallower, on Georges Bank. A more thorough mixing is characteristic of the latter group, a less pronounced thermocline and, as a consequence, a more nearly homogeneous water mass, top to bottom.

The data are given in Table II and plotted in depth-concentration curves, Figs. 2, 3, and 4. In the latter, the scale is different for each of the five items, and is so chosen as to make the curves most nearly correspond with each other. In the tables and curves the lower limit for nitrite nitrogen is taken as approximately 0.4 mg./m.^{3}; that is, the water is considered nitrite-free when amounts less than this are present, although with care it is possible to detect perhaps half this amount.

TABLE II

Hydrographic and Chemical Data for Stations in the Gulf of Maine and on Georges Bank

	Depth	Temp.	Salinity	Nitrate N	Nitrite N	Phosphate PO_4	Oxygen content	Oxygen saturation
	meters	° *C.*		*mg./m.*^{3}	*mg./m.*^{3}	*mg./m.*^{3}	*cc.*	*per cent*
Station 1329 August 2, 1932	1	19.19	32.00	25	0	18	5.40	98
	10	16.81	32.21	24	0	13	6.30	110
	20	11.02	—	35	0	11	7.27	113
	30	8.16	32.45	34	0	49	7.44	108
	40	6.2	32.63	120	1.3	77	6.63	94
	50	5.15	32.92	175	1.0	102	6.39	89
	60	5.2	32.79	195	1.0	100	6.34	88
	75	4.83	32.92	240	1.0	115	6.10	84
	100	4.59	33.19	275	0.8	85	5.79	80
	240	6.30	34.23	425	1.1	96	4.38	63
Station 1330 August 2, 1932	1	19.81	32.70	21	0	20	5.56	102
	10	19.43	32.20	21	0	11	5.57	102
	19	13.11	32.54	25	0	11	6.99	114
	29	8.80	32.66	27	0	36	7.26	109
	39	6.03	32.92	105	1.6	—	6.75	96
	48	5.01	32.92	170	0.4	43	6.51	90
	63	4.65	32.92	190	0.4	45	6.46	89
	77	4.45	33.01	240	0.7	54	6.34	87
	101	4.47	33.12	250	0	59	6.17	85
	189	5.81	34.23	390	0	82	4.76	68
Station 1331 August 3, 1932	1	16.57	32.75	28	1.2	12	6.07	106
	10	13.87	32.39	27	1.3	8	6.63	110
	19	9.65	33.03	35	2.1	9	7.24	111
	29	8.54	32.77	75	2.9	18	6.53	97
	39	6.79	32.92	135	3.6	17	6.07	87
	48	5.81	32.92	150	1.7	18	5.94	83
	58	6.00	33.12	210	1.4	19	5.78	82
	72	5.68	33.13	225	1.2	80	5.67	80
	97	5.53	32.97	285	0.9	—	5.43	76
	194	6.67	34.37	350	1.6	—	4.47	65
Station 1332 August 3, 1932	1	17.67	32.48	22	0	22	5.90	104
	9	17.60	32.72	20	0	22	5.90	104
	19	13.90	32.34	16	0	23	6.66	110
	28	11.09	32.66	27	1.5	36	6.95	107
	38	9.67	32.57	27	1.4	39	6.81	104
	47	8.65	32.59	60	2.7	50	6.82	102
	56	7.31	32.84	155	3.8	67	6.40	93
	71	6.13	33.68	235	1.5	86	5.94	84
	94	6.13	33.89	265	1.2	100	5.39	77
	188	7.17	34.85	450	1.8	132	4.27	63
	310	6.33	34.85	500	1.1	138	4.48	64

TABLE II (*Continued*)

	Depth	Temp.	Salinity	Nitrate N	Nitrite N	Phosphate PO_4	Oxygen content	Oxygen saturation
	meters	° *C.*		*mg./m.*^{3}	*mg./m.*^{3}	*mg./m.*^{3}	*cc.*	*per cent*
Station 1333 August 4, 1932	1	17.31	32.43	16	0	22	5.78	102
	9	16.81	32.34	20	0	28	6.00	104
	17	9.96	32.48	20	0	32	6.92	106
	26	7.57	——	80	3.5	36	6.68	98
	35	6.76	32.70	110	2.5	54	6.60	94
	44	6.10	32.83	150	1.7	73	6.40	90
	52	6.00	33.44	175	0.9	70	6.39	91
	65	5.61	33.44	240	3.7	81	5.94	84
	87	6.00	34.02	275	2.7	97	5.39	77
	186	6.67	34.90	460	3.7	100	4.69	68
Station 1334 August 4, 1932	1	14.98	32.52	20	0	25	6.10	103
	9	15.02	32.48	27	0	34	6.11	103
	17	14.15	32.66	24	0	38	6.12	102
	26	13.25	32.70	40	1.8	43	6.17	101
	35	12.55	32.70	45	1.9	35	6.24	101
	43	11.86	32.74	60	2.0	37	6.22	99
	52	9.01	32.79	100	2.5	38	6.28	95
Station 1335 August 4, 1932	1	15.50	32.54	25	1.9	26	6.34	108
	10	15.45	32.38	25	2.6	—	6.35	108
	20	12.90	32.74	25	1.4	—	6.68	109
	30	10.61	32.88	65	4.0	71	6.24	97
	40	10.04	32.79	80	4.1	64	6.18	95
	50	10.26	33.01	80	3.9	67	6.18	96
	60	10.11	32.74	85	4.0	62	6.17	95
Station 1336 August 5, 1932	1	15.23	32.70	21	1.0	31	6.25	106
	10	14.35	32.66	22	1.2	32	6.31	106
	20	11.07	32.70	60	3.2	35	6.22	98
	30	10.70	32.66	70	3.9	45	6.12	95
	40	10.65	32.68	70	3.5	37	6.06	94
	50	10.63	32.66	70	3.4	—	6.17	96
	60	10.63	32.74	75	3.9	63	6.10	95
Station 1337 August 5, 1932	1	19.85	32.95	26	1.6	29	5.82	107
	10	17.97	32.63	24	1.1	31	6.12	109
	20	11.49	32.68	24	0.6	28	7.24	114
	30	9.13	32.81	95	7.4	76	5.96	90
	40	8.98	33.44	105	7.4	69	5.95	90
	50	8.97	32.83	105	6.9	77	5.95	90
	60	8.97	32.83	105	7.0	82	5.89	89

Oxygen.—The figures for dissolved oxygen content agree in general with those found in other similar investigations. When the " percentage of saturation " is calculated it is found that the upper 20–40 meters are invariably " supersaturated," with a maximum corresponding to the level of greatest diatom abundance. This is brought out in detail in the section of this report dealing with diatom distribution. In no case is there any clearly defined minimum at an intermediate depth, but always a continuously decreasing percentage to the bottom.

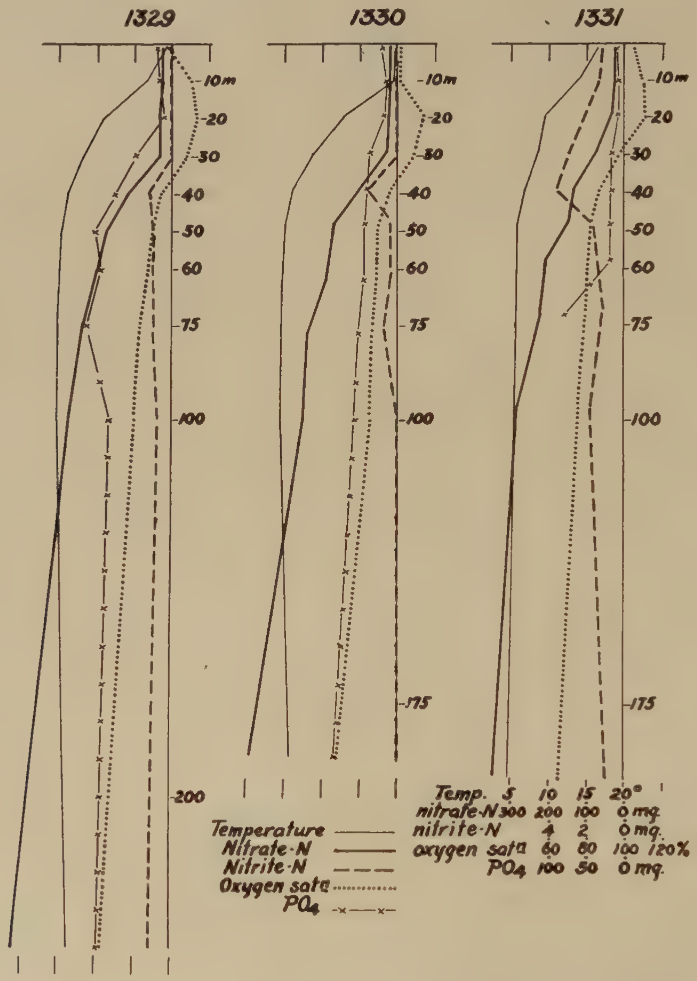

FIG. 2. Chemical data for stations in the deep basin of the Gulf of Maine, August, 1932.

Phosphate.—The phosphate values were obtained under rather less than ideal conditions but are satisfactory for comparative purposes. Only at the first three stations do the concentrations approach critically low values, and it is to be observed that the minimum quantities are found at depths of 10–20 meters, corresponding to the regions of highest oxygen saturation.

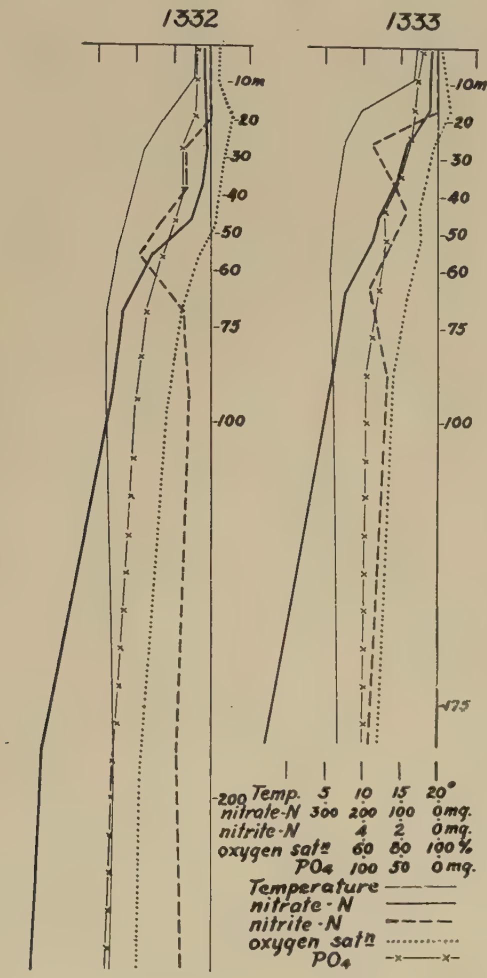

FIG. 3. Chemical data for stations near the Eastern Channel, August, 1932.

Nitrate.—The concentrations of nitrate nitrogen do not in any instance approach what have been considered minimal quantities. However, the correlation of oxygen saturation and diatom abundance with nitrate is much closer than with phosphate. The depth-concentration curves indicate how almost every change in oxygen is accompanied by a similar one in nitrate. The concentrations of nitrate reported here will be observed to be somewhat higher than those generally recorded in the literature. But four years of experience with the method leads the author to believe them fairly reliable. Each sample was determined in duplicate, and the color developed by the reagent compared with a set of dye-standards twice standardized against a series of sea-water samples with known amounts of added nitrate. Deep water samples were diluted with surface water of known nitrate concentration to bring them within the most reliable range of the standards. Anyone who has worked with the method will realize the uncertainties involved in determining absolute amounts of nitrate, although relative values are doubtless satisfactory.

Nitrite.—The nitrite determinations made on this cruise are but one part of a larger study of the variations and general significance of nitrite in the sea, which will be reported later. The position which nitrite occupies in the general nitrogen cycle in the sea has been pointed out on several occasions (9) (10) (7) (11 and others). Its occurrence in sea water, under many circumstances at least, is altogether certain, notwithstanding Harvey's doubts, expressed in 1926 (7). Since it occupies an intermediate position in the oxidation of ammonia to nitrate, its concentration might naturally be expected to be exceedingly variable, depending upon conditions. In fact, there are several agencies, both chemical and biological, which can alter it. The results of Atkins (11) and several others, including the present author, show conclusively that the presence of nitrite is not in any high degree dependent upon prox-

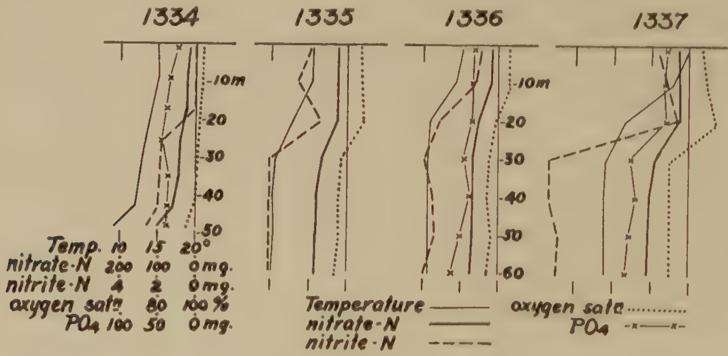

FIG. 4. Chemical data for stations on Georges Bank, August, 1932.

imity to the shore, even in cases in which pollution may be expected. Atkins' assumption (11) as to the significance of the nitrite concentration (namely, that it is an index of the extent of the nitrification process) is hardly adequate. That this is too simple an explanation of its variations may be seen even from the few data here reported, and more particularly on consideration of the bacteriological results in a later section of this report.

Under what may be considered normal conditions at this time of year, the sea water is devoid of nitrite in the upper levels. In only one of the five deep stations, No. 1331, do we find an exception to this, and this station, somewhat isolated in the northern basin of the Gulf, differs from the other stations in further respects. Its thermocline is not so pronounced, and the low phosphate values extend further down. When nitrite is found at the surface it is usually associated with rather large amounts lower down and with evidences of vertical mixing. This can be seen in the depth-concentration curves for Stations 1334–1337.

Another feature of the present investigation, which has been observed by the author on a number of other occasions, is the occurrence of a maximum nitrite concentration somewhere in the neighborhood of 40 meters. The finding of such intermediate maximum values and Waksman's establishment of the fact that the majority, if not all, of the oxidation of ammonia takes place in the bottom, show the inadequacy of regarding nitrite as a mere index of nitrification. Furthermore, Waksman's discovery of nitrate-reducing organisms in the upper levels, and their relation to the diatoms, make it very likely, as will be shown in the third paper of this series, that the nitrite maximum is due to reduction of nitrate rather than to oxidation of ammonia.

The actual bottom water differs in nitrate and nitrite content from that obtained in the lowest sample bottle. The water brought up with sand in the Peterson Grab at Station 1336 was filtered off and found to have a concentration of nitrate nitrogen of 350 mg./m.^{3}, and of nitrite, 25 mg. This may be compared with the nitrate, 75 mg., and nitrite, 3.9 mg., found in the sample at 60 meters, only four meters above. Approximately the same ratio between nitrate and nitrite exists in the two cases, but the enormous difference in the absolute quantities shows clearly where the active nitrification is going on. Again, mud obtained from Station 1331, when washed with nitrite-free surface water, yielded nitrite to the extent of 8.8 mg./m.^{3}, as compared with 1.6 mg. in the lowest water sample. Other similar cases from different areas will be found in a subsequent paper on this general problem.

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Contribution No. 9

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STUDIES ON THE BIOLOGY AND CHEMISTRY OF THE GULF OF MAINE

II. Distribution of Phytoplankton in August, 1932

H. H. GRAN[1]

The general distribution of the species of phytoplankton in the Gulf of Maine is well known from Bigelow's papers (1915, 1928). The summer conditions are described (1928, p. 391) as follows:

" In midsummer we have usually found the entire basin of the gulf occupied by a peridinian (Ceratium) plankton, with only occasional diatoms, and we have never found diatoms in abundance anywhere in the gulf in July or August except close along the coast, on the one hand, and on Georges Bank, on the other.

" We found diatoms flowering in abundance on each of our summer visits to the latter locality, but in different regions in different years. Thus they dominated on the western end of the bank on July 9, 1913, and on July 23, 1916, but when we visited that general locality on July 20, 1914, the water contained very few diatoms but, instead, a characteristic peridinian plankton. Three days later, however, we encountered a rich flowering of diatoms near the northeastern edge of the bank. Furthermore, the Georges Bank flowerings of July, 1913 and 1914, though far apart geographically, were both dominated by Guinardia. In July, 1916, however, we found no Guinardia on a traverse of the western part of the bank but swarms of Thalassiothrix and Rhizosolenia in its stead."

. . . " It is not yet clear whether any particular region on the banks is more favorable for the multiplication of diatoms than another, except that we have always found these rich flowerings on its shoaler parts and never close enough to the continental slope to be within the influence of the high temperature outside the edge, which in its own turn, supports various oceanic diatoms in small numbers mingled with peridinians of similarly Tropic origin."

During the cruise with the " Atlantis " in the beginning of August, 1932, we visited the same localities and found on the whole the same general distribution of the communities as in the previous years. The newer methods allowed us to study quantitatively the horizontal and vertical distribution of the various species in correlation with chemical and physical factors, and thus to supplement the previous observations

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[1] Contribution No. 9, Woods Hole Oceanographic Institution.

At each station water samples for centrifuging were collected from the same water bottles giving samples for chemical determinations. To each sample of 150 cc. were added 5 cc. of a neutralized formalin solution (equal parts of *pure* 40 per cent formalin and distilled water, neutralized by adding drops of a strong sodium carbonate solution to neutral reaction controlled by litmus paper, and carefully filtered before use). Of each sample 55 cc. were centrifuged, and calculated as 50 cc., because experience has showed that about 10 per cent of the content is lost as adhering to the glasses of the centrifuge and to the pipette, even if these are carefully cleaned by rinsing with chromic acid between each operation. The sediments of the four tubes of the centrifuge were carefully transferred to one tube with acute bottom and centrifuged once more. The clear water above the sediment was sucked off cautiously by a pipette and only a sufficient quantity left to be covered by a cover-glass 20 mm. square. The slide is ruled across with 0.4 mm. distance between the lines. For the counting a 4 mm. apochromate (Zeiss) was used. With a compensation ocular No. 4 two intervals are covered by the field of the microscope, with ocular 8 just one (0.4 mm.). The whole content below the cover-glass must be counted, as it is impossible to get the sediments evenly distributed. Every cell is counted also of species forming chains. When one or two chain-forming species are very abundant, it may be sufficient to count the number of chains and calculate the number of cells by multiplication with the average number of cells per chain, separately determined in that same sample. In the tables the frequency of each species is given as cells per liter, the figure of 20 thus representing one cell observed in 50 cc. of sea water.

Vertical hawls with No. 20 silk nets were taken at all stations and used for a preliminary investigation. As a rule all species observed in the net samples were also found in the centrifuged water samples except larger forms as e.g. *Acanthometron,* which was abundant at some of the stations in the deeper part of the Gulf, as also found by Bigelow in 1914. Further a number of nannoplankton species were observed which could not be caught by the nets.

Of the eight stations from which phytoplankton samples were examined, the first five (1329–1333) were taken in the deeper part of the Gulf of Maine, the last three (1334–1336) on Georges Bank. The two groups are best described separately, as they show considerable differences in the quantitative distribution of the species.

The positions of the stations and the data concerning the chemical condition of the sea water are given in the preceding paper (Rakestraw, 1933).

The Deep Basin of the Gulf of Maine

All stations in this area show a marked stratification with regard to the hydrographical conditions as well as to the vertical distribution of the plankton. The surface is covered, downwards to 10 meters or more, by a layer of high temperature, mostly above 15°, and low specific gravity (σ_t less than 25). These surface layers were less saturated with oxygen than the transition layers in the depths of 20–30 meters, which indicates that the photosynthesis of the phytoplankton had been more active at 20

Table I

"Atlantis" Station 1330, August 2, 1932

Depth, *meters*	1	10	19	29	39	48	77
Temperature, ° *C*	19.81	19.43	13.11	8.80	6.03	5.01	4.45
Oxygen, *cc. per liter*	5.56	5.57	6.99	7.26	6.75	6.51	6.34
Oxygen, *% of saturation*	102	102	114	109	96	90	87
Nitrate, *mg. N per cu. m.*	21	21	25	27	105	170	240
	cells per liter	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*
Halosphæra viridis juv.	—	—	340	—	—	—	—
Coccolithus pelagicus	220	140	—	—	—	—	—
Pontosphæra Huxleyi	260	220	6640	37200	300	—	—
Dictyocha fibula	—	—	20	80	—	—	—
Actinocyclus Ehrenbergi	—	—	20	—	180	160	120
Corethron hystrix	—	—	—	40	—	20	—
Coscinosira Œstrupi	—	140	—	220	720	160	60
Nitzschia seriata	—	—	40	600	280	—	—
Rhizosolenia alata	40	120	180	160	—	60	—
Rhizosolenia Shrubsolei	—	—	—	40	160	—	—
Ceratium bucephalum	—	—	—	—	140	460	60
Ceratium fusus	20	40	240	360	20	—	—
Ceratium longipes	20	—	20	20	60	280	80
Ceratium tripos	20	—	—	—	—	—	—
Exuviælla baltica	40	100	120	100	—	—	—
Gonyaulax orientalis	20	—	—	40	—	20	—
Gonyaulax orientalis, cystæ	20	40	240	660	40	80	20
Acanthostomella norvegica	—	—	—	20	20	—	—
Labœa conica	20	20	—	—	—	—	—
Labœa strobila	40	60	—	—	—	—	—

The following species were found in small numbers:

Cerataulina Bergonii 30 m., 20. *Chætoceros decipiens* 50 m., 60. *Coscinodiscus centralis* 30 m., 20. *C. excentricus* 30 m., 40. *Leptocylindrus danicus* 30 m., 40. *Nitzschia delicatissima* 30 m., 100. *Thalassiosira decipiens* 40 m., 40. *T. gravida* 30 m., 40.

Oxytoxum gracile 20 m., 20. *Peridinium brevipes* 10 m., 20. *P. Steinii* 10 m., 20. *Protoceratium reticulatum* 30 m., 20.

Favella denticulata 75 m., 20. *Lohmanniella spiralis* 10 m., 20. *Ptychocylis obtusa* 30 m., 20.

TABLE II

"Atlantis" Station 1331, August 3, 1932

Depth, *meters*	1	10	19	29	39	48	72
Temperature, ° *C*	16.57	13.87	9.65	8.54	6.79	5.81	5.68
σ_t	23.91	24.22	25.49	25.47	25.83	25.96	26.13
Oxygen, % *of saturation*	106	110	111	97	87	83	80
Nitrate, *mg. N per cu. m.*	28	27	35	75	135	150	225
	cells per liter	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*
Coccolithus pelagicus	—	—	—	—	20	20	20
Pontosphæra Huxleyi	2060	940	80	—	—	—	—
Distephanus speculum	—	—	—	120	—	40	—
Actinocyclus Ehrenbergi	—	—	—	40	20	20	—
Cerataulina Bergonii	—	—	160	280	—	—	—
Chætoceros borealis	—	—	—	—	160	20	—
Chætoceros compressus	—	—	—	1680	1060*	20*	40*
Chætoceros constrictus	—	—	320	3460	2700*	60	—
Chætoceros decipiens	—	—	—	360	500	40	140
Chætoceros diadema	—	—	—	700	200	—	—
Chætoceros laciniosus	—	—	—	440	160	—	—
Chætoceros radicans	—	—	—	—	80*	—	40*
Corethron hystrix	—	—	—	180	20	—	—
Coscinodiscus excentricus	—	—	—	—	40	20	—
Coscinosira Œstrupi	—	480	60	1800	1520	380	—
Leptocylindrus danicus	—	120	—	120	360	—	—
Rhizosolenia alata	1480	1780	700	200	220	20	20
Rhizosolenia Shrubsolei	—	—	100	200	120	—	—
Thalassiosira gravida	—	—	20	40	20	—	—
Ceratium bucephalum	—	20	260	440	40	—	—
Ceratium fusus	—	20	500	280	—	—	—
Ceratium lineatum	240	—	—	—	—	—	—
Ceratium longipes	—	20	—	80	380	200	40
Ceratium tripos	20	20	280	20	—	—	—
Dinophysis norvegica	20	—	20	—	—	—	—
Exuviælla baltica	—	—	—	—	40	60	120
Gonyaulax orientalis	20	—	280	80	—	—	—
Gonyaulax orientalis, cystæ	—	180	1060	740	100	20	20
Peridinium brevipes	—	—	20	20	20	—	—
Peridinium ovatum	—	—	—	20	80	100	—
Peridinium pellucidum	—	—	—	60	—	20	—
Peridinium Steinii	—	20	120	180	20	—	—
Favella denticulata	—	—	20	20	20	—	—
Lohmanniella oviformis	—	—	20	20	20	—	—
Ptychocylis obtusa	—	—	—	80	60	40	—
Tintinnopsis sp	—	—	—	80	80	—	—

The following species were observed in small numbers: *Chætoceros affinis* 30 m., 140; *Ch. convolutus* 30 m., 60. *Ch. debilis* 40 m., 360. *Ch. teres*, resting spores 40 m., 80; *Coscinodiscus radiatus* 40 m., 20. *Rhizosolenia fragilissima* 30 m., 160; *Rh. styliformis* 50 m., 20. *Skeletonema costatum* 30 m., 300. *Thalassiothrix longissima* 30 m., 60.

Gonyaulax spinifera 10 m., 20. *Peridinium depressum* 75 m., 40. *P. pyriforme* 30 m., 40. *Acanthostomella norvegica* 20 m., 20.

* with resting spores.

to 30 meters than at the surface. This also agrees with the fact that the phytoplankton had its maximum of frequency just in the same depths, 20 to 30 meters, as shown by Tables I and II, in which the results of the countings are represented for two selected stations, 1330 and 1331.

The dominating species were *Ceratium fusus, Pontosphæra Huxleyi* and germinating cystæ of *Gonyaulax orientalis.* The diatoms were represented by *Rhizosolenia alata,* which also elsewhere as a rule accompanies the Ceratium-plankton during the summer as a subordinate part of the phytoplankton, and by the little *Coscinosira Œstrupi.* Also *Nitzschia seriata* and *Rhizosolenia Shrubsolei* occurred in small numbers. Neritic diatoms were scarce, and some of them had resting spores as a sign that they may be regarded as the last remains of a population from an earlier season. At Station 1331 they were found in greater numbers than elsewhere, with a distinct maximum at 30 meters depth and an accumulation of resting spores at 40 meters. Just at this station the warm surface layer was shallower than at the other stations, the temperature was below 10° from 20 m. and downwards, and the high nitrate content characteristic of the deeper layers was found right up to 30 meters depth (75 mg. N per cu. m.), just where these diatoms had their maximum. Also at this station the surface layer itself was somewhat richer than elsewhere in the Gulf; *Pontosphæra, Rhizosolenia alata,* and *Ceratium lineatum* had their maxima above 10 meters depth.

Table III

Ceratium bucephalum, August 2–5, 1932

Depth	Stations							
	1329	1330	1331	1332	1333	1334	1335	1336
meters	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*
1	—	—	—	—	—	—	—	20
10	—	—	20	—	—	—	—	—
20	20	—	260	—	20	—	—	—
30	20	40	440	—	—	—	20	—
40	60	140	40	—	—	40	—	—
50	100	460	—	—	—	40	—	—
75 (60)	—	60	—	—	—	—	—	—

At all stations in the Gulf *Ceratium bucephalum* and *longipes,* as shown by Tables III and IV, had their maxima below 30 m., at 40 to 50 m., where the conditions for photosynthesis could not be optimal, judged from the low oxygen content of the water. Only at Station 1331, where the limit between the surface layer and the deeper layers seemed to have been raised to a higher level, *C. bucephalum* had its maximum as high up as 30 m. These two species were dominating the phytoplankton in the same area one month earlier, at the beginning of July, as was observed by the International Passamaquoddy Fisheries Commission, and have probably sunk down after their period of growth.

The two stations 1332 and 1333, taken in the eastern part of the Gulf, where the water according to Bigelow's observations flows into the Gulf from the south, were distinctly poorer than the others. Single specimens of rare oceanic species, like *Oxytoxum reticulatum* at Station 1333, prove the oceanic influence. But at the same time one may be allowed to conclude that the rich Ceratium-plankton of the Gulf has gotten its specific character in the Gulf itself by propagation of the species adapted to the local conditions, even if some of them may have floated in from outside.

GEORGES BANK

The plankton collections taken at the three stations on Georges Bank, 1334 to 1336, all show a marked difference from those of the inner Gulf, but between them there is a great similarity. Table V shows as an example the observations from Station 1334.

TABLE IV

Ceratium longipes, August 2–5, 1932

Depth	Stations							
	1329	1330	1331	1332	1333	1334	1335	1336
meters	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*
1	—	20	—	—	—	—	—	—
10	—	—	20	—	—	—	—	—
20	—	20	—	—	—	—	40	—
30	—	20	80	—	20	—	20	—
40	60	60	380	—	—	40	—	—
50	—	280	200	—	—	—	20	—
75 (60)	—	80	40	—	200	20	—	—

The species dominating in the Gulf, the *Ceratium* species, *Gonyaulax orientalis, Pontosphæra Huxleyi, Coscinosira Œstrupi,* occurred also on the Bank, but in much smaller numbers. The plankton on the Bank was dominated by oceanic diatoms, first of all *Rhizosolenia alata* (Table VI), which at Station 1334 formed quite a dense population with a maximum of nearly 50,000 cells per liter.

Also, *Rhizosolenia Shrubsolei* and *Nitzschia seriata* occurred in fair numbers, up to 880 and 1440 per liter in nearly all the samples, and *Rhizosolenia styliformis* was found evenly distributed. The neritic diatoms were less abundant; only *Leptocylindrus danicus* and *Thalassiothrix nitzschioides* were found in most of the samples in numbers up to 1400 and 1240 per liter, numbers which are quite inconsiderable in comparison with the quantities of the same species often observed in coastal waters. *Guinardia flaccida,* which was found on the Bank by Bigelow in July, 1913 and 1914 as a dominant species, occurred also in

TABLE V

Atlantis Station 1334, August 4, 1932

Depth, *meters*	1	9	17	26	35	43	52
Temperature, ° *C*	14.98	15.02	14.15	13.25	12.55	11.86	9.01
σ_t	24.08	24.04	24.37	24.58	24.73	24.88	25.41
Oxygen, *% of saturation*	103	103	102	101	101	99	95
Nitrate, *mg. N per cu. m*	20	27	24	40	45	60	100
	cells per liter	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*
Coccolithus pelagicus	—	—	—	—	—	40	20
Pontosphæra Huxleyi	840	160	—	—	—	—	—
Dictyocha fibula	—	40	—	—	—	—	20
Actinocyclus Ehrenbergi	—	—	80	80	40	—	120
Coscinodiscus excentricus	—	—	40	80	—	—	40
Coscinosira Œstrupi	40	—	—	120	—	80	140
Guinardia flaccida	—	—	—	—	—	40	100
Leptocylindrus danicus	—	—	1240	320	480	880	160
Nitzschia delicatissima	—	160	240	400	200	480	40
N. seriata	120	680	560	1080	1160	1440	1060
Paralia sulcata	—	—	—	—	—	—	500
Pleurosigma Normani	—	40	200	80	40	40	60
Rhizosolenia alata	27880	29720	47920	49800	27360	30120	15860
R. Shrubsolei	560	120	400	880	760	200	460
R. styliformis	200	120	40	120	120	40	140
Skeletonema costatum	—	—	—	160	—	160	—
Thalassiothrix longissima	80	—	40	40	160	—	—
T. nitzschioides	280	440	1240	960	560	1200	860
Ceratium bucephalum	—	—	—	—	40	40	—
C. fusus	—	120	80	—	40	—	100
C. lineatum	—	40	—	—	80	—	60
C. longipes	—	—	—	—	40	—	20
C. tripos	40	—	—	—	—	—	20
Gonyaulax orientalis, cystæ	40	—	—	—	—	40	20
Favella denticulata	—	—	80	40	40	40	40
Helicostomella subulata	40	40	40	80	120	40	40

The following species were observed in small numbers:

Actinoptychus undulatus 10 m., 40; *Chætoceros compressus* 20 m., 320; *Leptocylindrus minimus* 20 m., 640; *Rhizosolenia setigera* 50 m., 80; *Dinophysis acuminata* 20 m., 40; *Peridinium brevipes* 50 m., 40; *Pyrophacus horologium* 40 m., 40.

Ptychocylis obtusa 50 m., 20; *Lohmanniella spiralis* 60 m., 20; *Tintinnopsis* sp. 60 m., 20.

1932 at all three stations, but only in the samples from the deeper layers (Table XIV). It was most abundant at Station 1336 with a maximum of 340 per liter in 50 meters depth. The neritic tintinnid *Helicostomella subulata* was found evenly distributed at all three stations in numbers up to 120 per liter.

Very characteristic is the regular occurrence of some littoral diatoms, *Pleurosigma Normani*, *Paralia sulcata*, and *Navicula distans*. Their distribution indicates with certainty turbulence of the waters by which material from a shallow bottom has been carried out to sea.

TABLE VI

Rhizosolenia alata, August 2–5, 1932

Depth	Stations							
	1329	1330	1331	1332	1333	1334	1335	1336
meters	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*
1	160	40	1480	—	80	27880	3080	440
10	220	120	1780	120	20	29720	4320	1750
20	100	180	700	280	20	47920	23620	4040
30	60	160	200	220	20	49800	12720	1660
40	140	—	220	120	40	27360	7660	2720
50	—	60	20	20	20	30120	8220	1720
75 (60)	—	—	20	—	—	15860	5720	660

TABLE VII

Pleurosigma Normani, August 4–5, 1932

Depth	Stations		
	1334	1335	1336
meters	*cells per liter*	*cells per liter*	*cells per liter*
1	—	—	20
10	40	20	25
20	200	20	120
30	80	40	60
40	40	120	160
50	40	40	60
60	60	40	220

It is also remarkable that on the Bank all the species mentioned above were evenly distributed from the surface to the bottom (Tables VI and VII). Such a situation can only have been produced by a strong vertical mixing of the waters, which is also indicated by the even distribution of the temperature and salinity. The diatoms show a slight maximum at all stations at the depths of 20 to 30 meters. Just at this depth the photosynthesis seemed to have its optimum in the Gulf and the same may have been the case on the Bank, and thereby the growth may have been stimulated. But the difference in numbers corresponds only to one or two cell divisions and thus the turbulence of the waters must have had a stronger influence on the vertical distribution of the diatoms than the factors regulating their growth. The higher temperature of the very surface layer, from 1 to 10 meters, seems to have been slightly unfavorable for the growth or perhaps for the floating power—because of the lower viscosity of the water—of most of the species, for instance *Leptocylindrus danicus* (Table VIII).

Table VIII

Leptocylindrus danicus, August 2–5, 1932

Depth	Stations							
	1329	1330	1331	1332	1333	1334	1335	1336
meters	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*
1	—	—	—	—	—	—	—	—
10	—	—	120	—	—	—	—	—
20	—	—	—	—	20	1240	1400	560
30	—	40	120	—	—	320	1340	440
40	—	—	360	—	—	480	580	420
50	—	—	—	—	—	880	300	380
75 (60)	—	—	—	—	—	160	640	240

A comparison between the Gulf and the Bank shows first of all that the same species are found over the whole area, but the quantitative and also the vertical distribution is different. The stratification in the inner Gulf has favoured the growth of the *Ceratia* and of *Pontosphæra* (Tables IX and X), accumulated by their active movements in the depths of optimal life conditions. On the other hand, the mixing of the waters on Georges Bank has brought into circulation sufficient quantities of nutrient salts to produce a strong growth of oceanic diatoms, *Rhizosolenia alata* and others, and the continued vertical mixing has caused their even distribution from surface to bottom. At the innermost station on the Bank, Station 1334, this population has reached a nearly maximal density for so large a species (Tables V and VI).

Neritic diatoms, which are as far as we know, the most rapid growers of the plankton of temperate seas, were quite scarce over the whole area, although single cells or chains were observed everywhere. The only locality where a slight accumulation of them could be observed, was Station 1331, where for some unknown reason the nitrate content was high right up to 30 meters depth, and just at this depth the diatoms had increased (Table II). Evidently they require a higher concentration of nutrient salts than the surface layers of the Gulf of Maine contained at this season.

TABLE IX

Ceratium fusus, August 2–5, 1932

Depth	Stations							
	1329	1330	1331	1332	1333	1334	1335	1336
meters	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*
1	80	20	—	—	—	—	—	40
10	220	40	20	20	60	120	40	20
20	420	240	500	40	40	80	100	40
30	620	360	280	—	—	—	—	20
40	—	20	—	—	—	40	—	—
50	—	—	—	—	—	—	—	—
75 (60)	—	—	—	—	—	100	—	—

TABLE X

Pontosphæra Huxleyi, August 2–5, 1932

Depth	Stations							
	1329	1330	1331	1332	1333	1334	1335	1336
meters	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*	*cells per liter*
1	240	260	2060	260	2720	840	40	—
10	3880	220	940	960	2120	120	80	80
20	15620	6640	80	860	10660	—	2680	—
30	2000	37200	—	580	4140	—	40	—
40	80	300	—	40	3060	—	20	20
50	—	—	—	40	80	—	—	—
75 (60)	—	—	—	—	—	—	—	60

CULTURE EXPERIMENTS

As a contribution to the question of the requirements of nutrition of the plankton diatoms some culture experiments were made with the same method as was used previously on the Norwegian coasts. A series of closed, glass-stoppered bottles containing sea water with known content of plankton were suspended in the sea, all under the same conditions of light and temperature, and with the addition of various nutrient salts. The increase of the diatoms was determined by counting. When the culture bottles were exposed only a few days, and the population in the bottles was not allowed to increase above the maximum density observed in the sea, about one million cells per liter, the cultures could be kept under quite normal conditions, and by use of a hand lens the cells or chains could be seen normally and evenly suspended in the water within the bottles.

The main question for the experiments arranged at Woods Hole was that of the possible influence of soluble iron compounds on the growth of the plankton diatoms. As it is now well known, most of the differences in the productivity of the various areas in the sea can be explained from the distribution of the nitrates and phosphates, acting as limiting factors for the growth of the plankton algæ according to Brandt's theory.

But we have also some indications that the quantities of soluble iron present in sea water may be insufficient to support a rapid growth, particularly of the neritic diatoms, and my working hypothesis is that the soluble iron compounds, washed out with "humus"-compounds from the shores, may give the explanation of the relative productivity of coastal waters in comparison with the open ocean.

In an experiment made on the Norwegian coast (Gran, 1931) I had found that the growth of the neritic diatom *Skeletonema costatum* was enormously stimulated by the addition of small quantities of garden soil extract, corresponding to 10 mg. soluble Fe per cu. m., to the oceanic water in which it was living. In this water, which contained sufficient quantities of nitrates and phosphates, no other nutrients gave, when added, any positive effect at all.

If the effect of the soil extract is caused by its content of soluble iron, it must be possible to replace the soil extract by other soluble iron compounds, as Carsten Olsen (1929) did in his experiments with *Lemna,* using ferri-citrate with positive results. Recently also Burk and his collaborators (Burk, Lineweaver, and Horner, 1932) have proved by exact experiments that the stimulating effect of soil extract on *Azotobacter* is caused by its content of soluble iron.

My previous experiments to replace the soil extract by other iron compounds were not very successful. Oxyhæmoglobin or ferrisaccharate gave no positive effect, and the effect of ferricitrate or ferrosulphate was too small in comparison with that of the soil extract, possibly because these compounds were too quickly decomposed or oxidized in sea water.

At Woods Hole Dr. Waksman kindly gave me an opportunity to test his synthetic ferri-ligno-proteid (Waksman and Iyer, 1932) which, according to his great experience, may be regarded as identical with the essential components of "humus" and which at least has the same biological and chemical reactions.

For the experiments clean, glass-stoppered bottles of 130 cc. inner volume were filled from the same container with sea water of known plankton content, and suspended in the harbor at a depth of 1.5 meters.

To the bottles was added:

(1) No addition (3 bottles).

(2) 1 cc. of a solution containing 0.1 per cent KNO_3 and 0.01 per cent NaH_2 PO_4 (3 bottles).

(3) The same as (2) + 1 cc. of garden soil extract (1 liter garden soil, boiled in autoclave with 1 liter distilled water, then filtered, and the filtrate sterilized in autoclave) (3 bottles).

(4) The same as (2) + 0.1 cc. of a solution of 100 mg. ferri-ligno-protein in 500 grams water (3 bottles).

TABLE XI

Culture Experiment 1. Woods Hole, July 23–26. Temperature, 20°–22°. Surface water taken outside the harbor.

Diatoms	Before experiment	Without addition (1)	Nitrate and Phosphate (2)	Nitrate, Phosphate, and Soil (3)	Nitrate, Phosphate, and Ferri-ligno-protein (4)
	cells per cc.	*cells per cc.*	*cells per cc.*	*cells per cc.*	*cells per cc.*
Chætoceros compressus	2	1	243	310	415
Leptocylindrus danicus	13	16	466	792	628
Nitzschia delicatissima	28	58	957	595	789

The first experiment was made on July 23 with surface water taken outside the harbor of Woods Hole. This water was rather rich in diatoms, and therefore the bottles were suspended for only three days, until July 26, and their content preserved with formalin for counting. The water originally contained per cubic centimeter 22 cells of *Rhizosolenia calcar avis*, 24 of *R. Shrubsolei*, 2 *Chætoceros compressus*, 13 *Leptocylindrus danicus*, 28 *Nitzschia delicatissima* and some other species in very small numbers. The two first-named species did not grow well in the bottles; for the other three the average number per cubic centimeter is given in Table XI.

The result is clear. Without addition of nutrients the diatoms had not propagated at all, but the addition of nitrate and phosphate, in quantities found under optimal conditions in nature, was enough to stimulate them to a growth about equal to the maximal growth observed for plankton diatoms, corresponding to seven divisions in three days for *Chætoceros compressus* and five to six divisions for the other species. The extra addition of soil extract or of the ferri-ligno-protein had no marked effect. If the stimulating effect of the soil extract depends upon its iron content, the conclusion must be that the harbor water used for the experiment contained more than enough of soluble iron, while at the same time its content of nitrate or phosphate has been the limiting factor for further growth of the diatoms.

The experiment needed to be repeated with more oceanic water which, according to the theory, should contain less soluble iron if these compounds are renewed by outwash from shore. Therefore, on the last night of the cruise of the "Atlantis," the fifth of August, a large water sample was taken from the surface off Nantucket Lightship and a new experiment arranged the next day. The same plan as before was followed, except that to the bottles of series (4) was also added $MnCl_2$, corresponding to 10 mg. Mn per cubic meter. The water was rather poor in plankton. It contained six cells per cubic centimeter of *Rhizosolenia alata* and 0.4 cells per cubic centimeter of *Nitzschia seriata*. *Leptocylindrus danicus* must have been present to the number of 0.1 cells per cubic centimeter, although it was not found by counting a sample of 10 cc. The character of the plankton was rather similar to what was found at Station 1336 on Georges Bank.

The species reacted in different ways to the addition of the nutrients. *Rhizosolenia alata*, which is an oceanic species, had in five days without extra nutrition grown to make up a population of about the same density which was found at the innermost station on Georges Bank, Station 1334. With nitrate and phosphate it had grown very much more. The increase corresponds to six cell divisions in five days, which I have found to be the maximal growth rate for this species. The extra addition of soil extract or ferri-ligno-protein did not give any further effect.

The typical neritic species, *Leptocylindrus danicus,* reacted otherwise. With addition of nitrate and phosphate alone it increased from one to 130, which corresponds to seven divisions in five days, one cell division more than *Rhizosolenia*. But with soil extract it increased to a number ten to eleven times higher, and with Waksman's compound and $MnCl_2$ twenty-five fold. With soil extract it averaged ten to eleven cell divisions in five days, and with ferri-ligno-protein eleven to twelve divisions. The effect of the synthetic "humus" seems to be quite similar to that of the soil extract.

Nitzschia seriata also showed some effect of the soil extract and of the ferri-ligno-protein, but less marked than *Leptocylindrus.* In its distribution it is not so strongly limited to the coastal waters as *Leptocylindrus.* It is one of the very few motile species among the plankton diatoms, which may perhaps give the explanation of its relatively good growth in the bottles without added nutrition.

We intended to continue the experiments and to use for comparison also ligno-protein without iron, but had no opportunity to get water of sufficiently oceanic character. A third experiment gave results parallel to those of the first, a maximal effect of nitrate and phosphate alone and no extra effect of the iron compounds.

The results published here are not conclusive and the experiments should be repeated when opportunity arises. The method is practicable where water with living plankton can be brought in from oceanic situations.

The question, whether the presence of soluble iron compounds in sufficient quantities can be a limiting factor to the growth of the plankton diatoms, and particularly of the species with the most rapid growth rates, can not be solved, of course, until we also have more data about the distribution of the soluble iron compounds in the sea.

TABLE XII

Culture Experiment 2. Woods Hole, August 6–11, 1932. Temperature 20–22°. Water taken off Nantucket Lightship on the night of August 5.

Diatoms	Before experiment	Without addition (1)	Nitrate, Phosphate (2)	Nitrate, Phosphate, and Soil (3)	Nitrate, Phosphate, ferriligno-protein, and Manganese (4)
	cells per cc.	*cells per cc.*	*cells per cc.*	*cells per cc.*	*cells per cc.*
Rhizosolenia alata..........	6	29	403	466	478
Nitzschia seriata..........	0.4	36	365	1109	769
Leptocylindrus danicus....	0.1	1	13	150	335

The diatoms were quite normal in all cultures; in some bottles they had even formed auxospores. The figures represent averages of three parallel cultures.

In collaboration with Dr. Waksman and Dr. Reuszer a preliminary experiment was also arranged to follow the relation of the marine bacteria to the plankton diatoms.

A series of bottles filled with sea water taken at the surface outside the harbor of Woods Hole were suspended in the sea in the same way as described above. To some of them was added nitrate and phosphate in the same relation as in Experiments 1 and 2, while the others were left without any addition of nutrients. Bottles were taken to be studied with regard to their content of plankton and bacteria after three, seven and twelve days. The water was rather rich in plankton beforehand, and the number did not increase in the bottles without nitrate and phosphate. As shown in Table XIII, the plankton in the fertilized bottles had increased after three days to a maximal density. Most of the species were still in good condition and normally suspended in the water. After seven days most of the species had decreased in number, and dead cells formed lumps at the bottom of the bottles.

The bacteria increased and decreased with the diatoms, as Waksman and his collaborators report (in the paper following this one). The high temperature may have been one of the causes accelerating the decrease of the plankton after the maximum. Waksman's interesting idea of the correlation between the plankton and the bacteria can perhaps be studied favorably in experiments like this, in which the water is poor in plankton from the beginning and therefore the conditions in the bottles can be kept normal for some days. When some of the diatoms begin to die too many unknown factors are introduced.

Table XIII

Culture Experiment 4. August 18–30, 1932. Nitrate and phosphate as in Experiment 1 (2).

Diatoms	Before experiment	After 3 days	7 days	12 days
	cells per cc.	*cells per cc.*	*cells per cc.*	*cells per cc.*
Chætoceros compressus	16	95	18	57
Coscinosira Œstrupi	11	62	6	3
Leptocylindrus danicus	11	86	5	0
Nitzschia seriata	65	468	65	31
Rhizosolenia calcar avis	2	0	0	0
Rhizosolenia delicatula	36	32	7	0
Rhizosolenia fragilissima	21	84	37	0
Rhizosolenia setigera	6	6	21	27
Rhizosolenia Shrubsolei	1	0	0	0
Skeletonema costatum	(0)	139	3	9
Thalassiothrix nitzschioides	2	16	0	0
Other species	6	38	16	38
Sum	177	1026	178	165

REMARKS ON THE SPECIES

Heterocontæ

Halosphæra viridis Schmitz is the only annual plant of the phytoplankton, as far as known. It has never been observed to occur in such an abundance as to be found in great numbers in the centrifuged water samples. Young specimens were found at Station 1330 at 20 meters depth in a number of 340 per liter, and at Station 1333 it was observed in all samples from the surface to 50 meters, with a maximum of 140 per liter at 20 meters. Single specimens were observed at Stations 1332, 1334, and 1336.

Coccolithophorida

Coccolithus pelagicus Wallich was observed at the three stations in the inner part of the Gulf (1329–1331) and at Station 1336 on Georges Bank. Its maximum of frequency, 220 cells per liter, was found at Station 1330 near the surface, 1–10 meters. At Station 1336 it occurred in all samples from 20 meters downwards, with a maximum of 100 at 40 meters depth.

Pontosphæra Huxleyi Lohmann is the most common of all the Coccolithophorida. Although it is an oceanic species it can occur in enormous numbers, millions per liter, in coastal waters also, where the temperature is sufficiently high. As the table shows, it was found over the whole area, distinctly more abundant at the deeper stations in the Gulf than on Georges Bank, with a maximum of frequency of 37,000 per liter at Station 1330.

Silicoflagellata

The few species of this order have a wide distribution but never occur in abundance.

Dictyocha fibula Ehrenberg was observed at two stations in the Gulf and at three stations over Georges Bank, nowhere in higher numbers than 80 per liter.

Distephanus speculum Ehrenberg is a more northern species, which was observed only at Station 1331, together with the maximum of neritic diatoms, most abundant (120 per liter) at 30 meters depth, and at Station 1336.

Diatoms

In temperate and arctic waters the diatoms make up the bulk of the phytoplankton where the nutrient salts are present in sufficient quantities. Some of them, particularly the neritic species, as *Skeletonema costatum, Chætoceros debilis, C. compressus,* and others, *Leptocylindrus danicus, Thalassiosira Nordenskiœldii,* are able to reproduce themselves under favorable conditions very quickly, with up to two cell divisions per day. Also some oceanic species, as *Rhizosolenia alata* and *R. semispina, Chætoceros decipiens, Nitzschia seriata,* and *N. delicatissima* can form very dense populations, but their propagation seems to be less rapid than that of the neritic ones mentioned above. Other species as, e.g., the large *Coscinodiscus,* can be very commonly distributed but never so abundant that more than a few specimens are found by centrifuging

50 cc. of sea water. A number of littoral species occur so regularly in the plankton that they have to be reckoned with as signs of turbulence of the water, although their number is quite insignificant in comparison with the true pelagic species. It is rather remarkable that only some few out of the great number of littoral species are found regularly in the plankton, as in our material *Actinoptychus undulatus, Navicula distans, Pleurosigma Normani,* and *Paralia sulcata.* They may perhaps even propagate to some extent in the floating condition.

Actinocyclus Ehrenbergii Ralfs. This species was observed at three stations in the central part of the Gulf (1329–1331) and at all stations on Georges Bank (1334–1336). Here it was found in all depths from 20 meters to the bottom in numbers of up to 300 per liter at Station 1336, at 30 meters depth. Its heavy, thick-walled cells are more often found in greater depths than in the surface layers, and it is in my opinion a characteristic representative of the " shade-flora " of Schimper (Karsten, 1905). Ostenfeld (1913) regarded it as a littoral, " tycho-pelagic " species.

Actinoptychus undulatus Ehrenberg was observed as single specimens at all stations on Georges Bank. It is without doubt a littoral species.

Cerataulina Bergonii H. Peragallo. This neritic species had a small maximum in 20 to 30 meters depth at Station 1331 (160 to 180 per liter) together with the neritic *Chætoceros* species. Single specimens were also observed at Stations 1330, 1332, and 1336.

Chætoceros. Eleven species of this genus were observed. All of them except the oceanic species *Ch. atlanticus* occurred and had their maximum at Station 1331 (see Table II) at 30 to 40 meters depth as remains of a more abundant growth earlier in the season. At 30 meters the high nitrate content of the water may have caused some increase, but at 40 meters many of the species had formed resting spores and were evidently sinking. At Station 1329 the situation was similar but less pronounced. Here only the two most common species, *Ch. compressus* and *Ch. constrictus* occurred in small numbers (120 to 260 per liter), the former with resting spores.

On Georges Bank the oceanic species *Ch. atlanticus* and particularly *Ch. decipiens* occurred at the two outer stations, 1335 and 1336, *Ch. atlanticus* very scarce in a few samples (60–80 per liter), *Ch. decipiens* in all samples except at the surface, in numbers up to 340 per liter at 20–30 meters at Station 1335. Here also the neritic species *Ch. compressus* and *laciniosus* were found in small numbers, mostly with resting spores.

The species found in the material were: *Ch. affinis* Lauder, *Ch. at-*

lanticus Cleve, *Ch. borealis* Bailey, *Ch. compressus* Lauder, *Ch. constrictus* Gran, *Ch. convolutus* Castracane, *Ch. debilis* Cleve, *Ch. decipiens* Cleve, *Ch. diadema* Ehrenberg, *Ch. laciniosus* Schuett, *Ch. radicans* Schuett, *Ch. teres* Cleve.

Corethron hystrix Hensen occurred in small numbers at Stations 1330 and 1331, with a maximum of 180 per liter at 30 meters depth at Station 1331.

Coscinodiscus. The centrifuge method cannot give a good picture of the distribution of the larger species of this genus, as the quantity of water (50 cc.) is too small. *C. centralis* Ehrenberg was observed at Station 1330, *C. radiatus* Ehrenberg at Stations 1331 and 1336, but only as single specimens. *C. excentricus* Ehrenberg was found more regularly, at all stations except 1329 and 1332. It seemed to be more common over Georges Bank than in the deeper parts of the Gulf, with maximum records of 80 per liter at 30 meters depth at Stations 1334 and 1336.

Coscinosira Œstrupi Ostenfeld occurred regularly at all stations, less abundant on Georges Bank than in the inner part of the Gulf, and with a maximum of abundance 1800 per liter at Station 1331, at 30 meters depth, together with the maximum of the neritic *Chætoceros* species. This little species of which Hustedt (1927–30, p. 317) recently has given excellent figures and description, is one of the very few representatives of the plankton of the western side of the North Atlantic, which is lacking, or at least very rare along the European coasts.

Guinardia flaccida H. Peragallo was dominating in the plankton of Georges Bank in July, 1913 and 1914 (Bigelow, 1928). It occurred also in August 1932 at all stations on the Bank, but not in great numbers, and with its abundance increasing toward the bottom, as shown by Table XIV. *Guinardia* was not observed in the deeper parts of the Gulf.

Leptocylindrus danicus Cleve (see Table VIII) occurred regularly at all stations on Georges Bank from 20 meters to the bottom, with maximum at 20 meters depth at all three stations. It was also found in small numbers at Station 1331 together with the *Chætoceros*-plankton and in traces at Stations 1330 and 1333.

Leptocylindrus minimus Gran was observed in small numbers, up to 640 per liter at Stations 1334–1336 on Georges Bank, at 20–30 meters depth.

Navicula distans W. Smith was observed as single specimens at Stations 1329 and 1335. At Station 1336 on Georges Bank it was found in all samples from 10 meters to the bottom. It is a littoral species with thick cell walls.

Nitzschia closterium W. Smith. Single specimens at Stations 1329 and 1336.

Nitzschia delicatissima Cleve occurred regularly at all depths from 20 meters to the bottom at Stations 1334–1336 on Georges Bank, but in very small numbers, up to 480 per liter. At Stations 1329 and 1330 traces were observed.

Nitzschia seriata Cleve occurred as well in the inner part of the Gulf as on Georges Bank, with a maximum of 1440 per liter at Station 1334.

Paralia sulcata (Ehrenberg) is another littoral species, which was found as scattered chains at Stations 1332–1336 in the deeper layers of water.

Pleurosigma Normani Ralfs was observed in nearly every sample from Georges Bank, rather evenly distributed from the surface to the bottom, as a sign of the turbulence. In a sample of 50 cc., from one to eleven cells were counted (Table VII).

TABLE XIV

Guinardia flaccida, August 4–5, 1932

Stations.......		1334	1335	1336
		cells per liter	*cells per liter*	*cells per liter*
Depth in meters	1	—	—	—
	10	—	—	—
	20	—	—	20
	30	—	—	160
	40	—	20	40
	50	40	140	300
	60	100	220	340

Rhizosolenia alata Brightwell was, in accordance with Bigelow's observations in previous years, the most common diatom at this season over the whole area and occurred at all stations, subordinate to the *Ceratia* in the stratified waters in the deeper part of the Gulf, but dominant on Georges Bank. Here it was distributed rather evenly from surface to bottom with a slight maximum at 20 to 30 meters depth. At Station 1334 at the inner side of the Bank the population was quite dense, with a maximum of nearly 50,000 cells per liter.

Rhizosolenia fragilissima Bergon (including *R. færoënsis* Ostenfeld), a neritic species which sometimes occurs in abundance, was observed only at Station 1331 at 30 meters depth in quite small numbers, together with the maximum of neritic *Chætoceros* species.

Rhizosolenia Shrubsolei Brightwell occurred together with *R. alata,* as it often does, but in much smaller numbers. On Georges Bank it was found evenly distributed from the surface to the bottom, in numbers up to 800–900 per liter. In the deeper part of the Gulf it was much less frequent and was limited to the layers between 20 and 40 meters depth.

Rhizosolenia styliformis Brightwell. This large and beautiful oceanic diatom had the same distribution as *Rh. Shrubsolei,* but occurred in smaller numbers. The highest frequency observed was 520 per liter at Station 1335 at 60 meters depth, but as a rule the number counted was only 200 per liter or less.

Rhizosolenia setigera Brightwell is a neritic species which was observed only in quite small numbers on Georges Bank, less than 100 per liter.

Skeletonema costatum (Greville). Single chains were observed at Stations 1331 and 1334.

Thalassiosira decipiens (Grunow) was observed quite rarely between 40 and 50 meters depth at the first two stations (1329–1330).

Thalassiosira gravida Cleve occurred at most of the stations but was quite scarce. The highest number observed was 140 per liter at Station 1329, 50 meters depth.

Thalassiothrix longissima Cleve and Grunow occurred rather evenly on Georges Bank, particularly at Station 1334, where it reached a maximum of 160 per liter at 40 meters. It is regarded as a subarctic oceanic species.

Thalassiothrix nitzschioides Grunow was only observed on Georges Bank but occurred there evenly distributed from surface to bottom in numbers from 200 to 1200 cells per liter.

Dinoflagellata

Ceratium. The species of this genus are by far the most important among the Dinoflagellata of the North Atlantic. They are oceanic, but are often found in greater abundance in semi-coastal areas as the North Sea, the Gulf of Maine, and the Gulf of St. Lawrence than in pure oceanic waters. Here they usually begin to dominate the phytoplankton as soon as the waters become stratified, after the spring growth of the diatoms has finished. They succeed each other according to their temperature requirements. *C. arcticum* and *C. longipes* dominate in the first part of the season, followed by *C. fusus* and *tripos.*

In the Gulf of Maine *C. fusus* was the dominating species in the beginning of August (Table IX) in the stratified waters. Its maximum of 500–600 per liter was found just at the depth where the values for saturation with oxygen were maximal and the life conditions optimal for the phytoplankton, at 20 to 30 meters depth. Two other species, *C. longipes* and *C. bucephalum,* were also rather abundant at the same stations (Tables III and IV) but their maxima were found deeper down, 40–50 meters depth, evidently below the depth of the optimal conditions for photosynthesis, as the water at these depths was not saturated with oxygen. Other observations, from the investigations of the International Passamaquoddy Fisheries Commission, show that these two species were dominating not far from the localities where our stations were taken, one month earlier, in the beginning of July. One may conclude that they have sunk into deeper layers after their main growth period was ended and they were succeeded by *C. fusus.* From my experiences on European coasts it is astonishing to find *C. bucephalum* early in the season together with *C. longipes,* as we used to find its maximum in September, together with that of *C. furca* which I have not observed at all in the Gulf of Maine.

Besides these species *C. arcticum* was found in small numbers at Station 1329, from 20 to 50 meters depth.

Ceratium tripos (O. F. Mueller) occurred at the same stations as *C. fusus,* and at the same depths, but less abundantly. Its maximum was found at 20 meters at Station 1331, with 280 per liter.

Ceratium lineatum (Ehrenberg) seems to be more neritic than the other species. It was found at the surface at Station 1331 in the number of 240 per liter, and on Georges Bank it occurred at all stations, rather evenly distributed with regard to the depth, with a maximum of 150 per liter at Station 1336, at 10 meters depth.

Dinophysis acuminata (Claparède and Lachmann) was observed as single specimens at two stations, 1329 and 1334.

Dinophysis norvegica Claparède and Lachmann was also quite scarce. Single cells were observed at four stations, in seven samples.

Exuviœlla baltica Lohmann occurred at all stations with its maximum near the surface. The highest number counted was 480 per liter at 10 meters depth at Station 1335. In coastal waters it can sometimes be found in much greater numbers.

Gonyaulax orientalis Lindemann. This species, described also by Miss Lebour (1925, p. 93) seems to be rather common. Rather few vegetative cells were found, but a number of globular thick-walled cysts, containing chromatophores and oil drops. Some of them were in cell division, and two naked cells escaped which developed into *Gonyaulax* cells. They were rather abundant in the inner part of the Gulf in depths from 10 to 30 meters, with a maximum of 1340 at Station 1331, but less common on Georges Bank. The diameter of the cysts was from 45 to 64 μ, the thickness of their cell wall 1.5 μ.

Gonyaulax spinifera (Claparède and Lachmann) was rare in the deeper part of the Gulf. At Station 1329 80 cells per liter were found as a maximum.

Oxytoxum gracile Gran (1929, p. 45). Single specimens were observed at two stations (1330 and 1332).

Oxytoxum reticulatum (Stein). One specimen of this rare oceanic species was observed at Station 1332 at 20 meters depth.

Peridinium

The marine species of *Peridinium* (exclusive of *Minuscula* Lebour) are heterotrophic and rarely occur abundantly enough to be regularly found in the centrifuged water samples. Eight species were observed: *P. achromaticum* Levander, *P. conicum* Gran, *P. brevipes* Kofoid, *P. depressum* Bailey, *P. ovatum* Pouchet, *P. pellucidum* Bergh, *P. pyriforme* Paulsen, *P. Steinii* Joergensen. Of these only *P. Steinii* and *P. ovatum* were found in a maximum abundance of 100 per liter or more, both of them at Station 1331, where seven of the eight species were found.

Protoceratium reticulatum Bergh, one specimen at Station 1329.

Pyrophacus horologium Stein. This neritic and autotrophic species was found at all stations on Georges Bank, but always in small numbers, up to 60 per liter.

Infusoria ciliata

Acanthostomella norvegica (Daday) was observed in small numbers at four stations in the deeper part of the Gulf, with the maximum of 80 per liter, at Station 1329, 20 meters depth.

Favella denticulata (Ehrenberg), the most common tintinnid in the collections, was found at all stations, mostly in 20 to 30 meters depth, with a maximum of 320 per liter at Station 1329, 20 meters depth.

Helicostomella subulata (Ehrenberg) is a neritic species which was found exclusively on Georges Bank, and here at all stations, with the maximum of 220 per liter at Station 1335, 20 meters depth.

Labœa Lohmann. The species of *Labœa* are not found in the net samples, but some of them which live in symbiosis with small brown cells can at times occur rather abundantly in the centrifuged water samples, particularly in spring together with the neritic diatom plankton. In August they were very scarce. *L. conica* Lohmann and *L. strobila* Lohmann were observed at Station 1330 from 1 to 10 meters depth, *L. acuminata* Leegaard and *L. vestita* Leegaard at Station 1335.

Lohmanniella oviformis Leegaard was observed in small numbers at four stations, 1331–1333 and 1336. At Station 1333 it occurred from 10 to 30 meters depth with a maximum of 60 per liter at 30 meters.

Lohmanniella spiralis Leegaard. Single specimens were observed at Stations 1330 and 1334.

Mesodinium rubrum Lohmann was quite scarce at this season. At Station 1335 on Georges Bank about 100 per liter were found at 1 to 10 meters depth, at Station 1333 a single specimen at 20 meters.

Ptychocylis obtusa Brandt was observed at Stations 1329–1331 in the Gulf and on Georges Bank at Stations 1334 and 1335, with a maximum of 80 per liter at Station 1331, 30 meters depth. A single specimen of *Tintinnopsis campanula* (Ehrenberg) was found at Station 1335.

Stenosemella sp. occurred on Georges Bank and at Stations 1332–1333 with a maximum of 100 per liter at Station 1335, 20 meters depth.

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STUDIES ON THE BIOLOGY AND CHEMISTRY OF THE GULF OF MAINE

III. Bacteriological Investigations of the Sea Water and Marine Bottoms

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In former expeditions undertaken for the study of marine life, the occurrence, abundance, and activities of the bacteria were either left out entirely, or were based upon the examination of samples of water brought back many days and frequently months after they were collected. The bacteriological activities in the sea bottom were frequently completely overlooked. In view of the fact that the total numbers of the bacteria as well as the relative abundance of specific forms will change rapidly soon after the samples of water and mud are exposed to surface temperature, due either to a change in environmental conditions or to a change in food supply, it is quite essential that the collected samples should be subjected to immediate investigation. As a result of these considerations, the laboratory on the "Atlantis," during the cruise made in August, 1932, was so outfitted that it was possible to carry out the bacteriological studies within a few minutes or at most hours, after the samples of water, bottom mud, or sand were brought up from their respective depths.

Because of the extensive hydrographic and biological investigations which have been carried on in the Gulf of Maine, this region is particularly well adapted to a study of the relative abundance and activities of marine bacteria. Such a study was further aided by the fact that other biological and chemical investigations were carried on simultaneously on this cruise. The locations of the various stations at which samples were taken during this cruise are described in the first paper of this series.[2] Of the eight stations studied, five were in the Gulf of Maine proper and the remaining three in the shallow waters over Georges

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[1] Contribution No. 10 of the Woods Hole Oceanographic Institution and Journal Series Paper of the New Jersey Agricultural Experiment Station.

[2] Rakestraw, Norris W., 1933. Chemistry of the Waters of the Gulf of Maine in August, 1932. *Biol. Bull.*, **64**: 149.

Bank. A detailed description of the methods used in these investigations will be presented later, when the bacteriological results of this expedition will also be compared with those obtained from waters and sea bottoms near shore. Only a brief summary of the investigations carried out on and in connection with this cruise of the " Atlantis " will be reported here. An attempt will be made to interpret the results of these investigations in the light of the chemical conditions of the waters and nature of the marine bottom, as well as to correlate the results of the bacteriological investigations with the results of the chemical and planktonological studies made by the other investigators on this cruise.

Abundance of Bacteria in Sea Water and in the Sea Bottom

The relative numbers of bacteria in the sea are usually determined by means of the plate method. This method has numerous limitations and can at best give only a very approximate idea of the actual abundance of the bacteria, both in the water and in the sea bottom. Although new methods have been introduced recently, consisting in direct staining of the bacteria in the water, after they have been concentrated by filtration or centrifuging, these methods are still in an experimental stage and were not used on this expedition. If the various limitations of the plate method are kept in mind and if broad generalizations are avoided, the results obtained even by this procedure can be made to throw considerable light upon the relative abundance and nature of the bacterial population of the sea.

The numbers of bacteria were determined in the water from the different stations at three different depths, namely, the surface, 50 meters, and the bottom. At one station, water from two additional depths of 30 and 100 meters was also investigated. The surface samples were taken with ordinary sterilized and evacuated glass tubes provided with a sealed glass tip which was broken about one foot below the surface of the water. Deeper samples were taken in heavier-walled glass tubes having a specially constricted neck to prevent the rubber stopper being forced into the tube by the pressure of the water. The inlet tube in this case was made of a capillary glass tube having a 1 mm. bore. Tubes of this type were used successfully to a depth of 330 meters but the lower limit of their usefulness has not yet been determined. Mud samples were taken by means of a sampler consisting essentially of a brass tube carrying a weight sufficient to force it some distance into the mud when the bottom is reached. Into the brass tube is fitted a glass tube having a check valve attached to its upper end. This allows water to stream through the tube during its descent. While raising the sampler the valve closes and prevents any downward pressure upon the mud core within. By sterilizing the glass tube before use it is possible to eliminate any bacterial contamination of the mud sample other than the contamination of the walls of the glass tube by the water passing through it while descending. This may be easily eliminated by taking the sample to be studied from the center of the core and discarding the surface which has been in contact with the glass tube.

The water was plated undiluted and diluted 10 times, while the mud was plated in dilutions of 1:10, 1:100 and 1:1000. Previous experiments, which will be reported elsewhere, have shown that the following medium is satisfactory for the development of marine bacteria, capable of growing on the ordinary plate:

Agar	15 grams
Glucose	1 gram
Peptone	1 gram
K_2HPO_4	0.05 gram
Sea water	1000 cc.

TABLE I

Abundance of bacteria in sea water. Total numbers in 1 cc. of water.

Station Number		1329	1330	1331	1332	1333	1334	1335	1336
Total depth of water, *meters*		256	207	230	346	230	71	74	64
Depth of sample, *meters*	Surface	10	3	4	2	380	16	92	6
	30			0					
	50	1	1	2	1	2			
	100			1					
	Bottom	6	0	1	1	3	25	41	27

The plates were incubated at air temperature and the colonies counted at the end of 7 and 10 days. All the samples of water and mud were plated out within a few minutes after being taken.

The total numbers of bacteria in the water, as determined by the plate method, are given in Table I. With respect to their bacterial content, the stations may be divided into two groups, the first containing the five deep water stations and the second the three shallow stations. With the exception of the surface water of Station 1333, the bacteria in the water of the first group, namely, Stations 1329 to 1333 inclusive, were uniformly so extremely low in number, that it is difficult to draw reliable conclusions in regard to their distribution. It would seem, however, that the bacteria were slightly more numerous at the surface than in the lower depths of water. There was no appreciable variation between the different other depths sampled, either at a single station or between different stations. Neither was there any appreciable variation in the bacterial content of the surface water of the different stations of this group. The very high content of the surface water of Station 1333 was probably due to the sample inadvertently being taken near floating seaweed or the introduction of some other contamination. The water samples from the lower levels of this station resembled those from the other deep water stations in their bacterial content.

The bacteria in the water of the second group of stations were uniformly much more numerous than in the water from the deeper stations. In view of the other results to be reported below, this may be ascribed to the higher phytoplankton content of this water. In two of the three stations the bottom water samples were considerably higher in bacteria than the surface samples. One must keep in mind the fact that the bottom of the last group of stations was sandy, while the bottom of the first four stations was mud. To what extent the deposits at the bottom of the ocean directly affect the bacterial content of the water for any very great distance above, except in turbulent waters, still remains to be determined. Because of the stray of the sampling cable from the perpendicular and the rise and fall of the ship with the waves, it is not possible to secure water samples with the apparatus used nearer than 2 or 3 meters from the bottom. The results, therefore, cannot be taken as indicating the bacterial content of the layer of water in contact with the mud bottom. It is possible to secure water samples nearer the bottom at shallow stations, as in the case of the second group. This, together with the fact that the water at these stations is rather turbulent, may indicate the transfer of sufficient nutrient material from the bottom deposit to the water immediately above to support the greater number of bacteria found in these waters.

The more uniform distribution of the bacteria in the water at the shallower stations is also correlated with the even distribution of the diatoms. This is probably due to the stronger vertical mixing of the waters, as shown by the even distribution of temperature and salinity.

The results reported above agree with those of various other investigators in indicating that the numbers of bacteria in sea water are relatively low and quite uniform in distribution. In an effort to secure further information concerning the presence of bacteria in sea water, the association of these organisms with the plankton forms was investigated. By towing with plankton nets samples of both zoöplankton and phytoplankton were collected. A number 20 silk net was used, which left out the nannoplankton. The zoöplankton consisted largely of copepods, while the phytoplankton was made up almost entirely of diatoms with a few *Ceratium* cells present. This material was collected in the glass jar at the bottom of the net and portions of it immediately plated out. The numbers of bacteria in the various tow waters are given in Table II.

The water containing the diatom plankton is extremely rich in bacteria. It was not possible to make determinations of the actual quantity of plankton present in the samples. Apparently the zoöplankton has fewer bacteria associated with it than the phytoplankton. Dr. Gran estimated that the sample obtained from Station 1336 contained 11,900 planktonic organisms per liter of tow water, of which 11,100 were diatoms. It is of considerable interest to compare the relative numbers of diatoms in the tow and in the sea water at this station with the numbers of bacteria in the tow water and in the sea water. In the case of the zoöplankton (Station 1331), there were 225 times as many bacteria in 1 cc. of the tow as in 1 cc. of the corresponding water, thus giving a ratio of the bacteria in the tow to the bacteria in the water of 225 : 1.

TABLE II

Abundance of bacteria in tow water. Numbers in 1 cc. of tow.

Station No.	Type of plankton	Total bacteria	Agar-decomposing bacteria	Percentage agar-decomposing bacteria
1331	Zoöplankton	890	0	0
1335	Diatom plankton	36,700	2,100	5.7
1336	Diatom plankton	37,500	2,500	6.7

In the case of the diatom plankton, the ratio between the bacteria in the tow and the bacteria in the water (taking the average of the surface and bottom samples) was 500 : 1 and 2,270 : 1, for Stations 1335 and 1336 respectively. It is important to note that Dr. Gran found that the ratio for the respective numbers of diatoms in the tow and in the water was about 1,667 : 1. This indicates that a definite parallelism exists between the bacteria and the diatoms in the water and in the plankton. The actual existence of such relations is substantiated by the fact that the bacteria from the diatom tow showed a considerable abundance of agar-liquefying organisms. While one encounters only infrequently such organisms in the free water, usually only very near shore, and in the mud, they were found to make up 5.7 and 6.7 per cent of the total bacterial flora in the plankton tow, as determined by the agar plate method. It is interesting to note that the bacteria found in the copepod-plankton did not include any agar-liquefying forms.

It is quite possible, therefore, that bacteria exist only to a very limited extent in the free water of the sea, but are largely attached to the plankton organisms, living upon the dead members of the plankton or upon the excretion products of the cells, upon the cell membranes, especially the mucilaginous substances secreted by certain algæ, etc. One need not, of course, imagine that if such an associative growth exists, the numbers of higher plankton forms and bacteria will always be parallel. One can readily imagine that the bacterial maximum may be attained after the diatom and copepod maximum. This has been brought out in an experiment carried out in cooperation with Dr. Gran and described by him elsewhere, in which the numbers of diatoms and of bacteria were determined simultaneously in artificial cultures of diatoms in sea water (Table III). The rise in numbers of both groups of organisms appears to have been directly related. The increase in bacterial numbers was much greater proportionately than that of the diatoms. When the multiplication of the latter reached a maximum, the numbers of bacteria continued to rise further, while the numbers of diatoms began to fall. This may be noted at the 7-day period when the diatoms had dropped to their original level, while the bacterial numbers were still increasing. This is to be expected since the death of the diatoms provides a source of food for the saprophytic bacteria. The numbers of bacteria dropped slowly and, within the course of the experiment, did not fall to the level of the original water as did the diatoms, indicating a greater resistance of bacteria to the artificial culture conditions.

TABLE III

Comparative numbers of diatoms and bacteria in diatom cultures. Numbers in 1 cc. of culture water.

	Original water	After 3 days growth	After 7 days growth	After 12 days growth
Diatoms........	177	1,026	178	165
Bacteria........	346	20,600	22,800	13,900

Because of the large numbers of bacteria capable of decomposing agar found associated with the diatoms of Stations 1335 and 1336, it was of interest to follow their behavior in this experiment. In the original water the number of agar-decomposing bacteria was of the order of 2 or 3 cells per cubic centimeter. At the end of the 3-day culture period this figure had risen to 50 cells per cubic centimeter. At none of the later periods did any agar-decomposing bacteria appear on

Table IV

Abundance of bacteria in the sea bottom. Numbers in 1 gram of mud or sand, dry basis.

Station Number	1329	1330	1331	1332	1335	1336
Surface	5,400	4,700	830	3,000	1,280	670
10 cm	1,720	1,700	140	290		

the plates. The disappearance of these bacteria may be ascribed to their overgrowth by other forms better adapted to the cultural conditions. This is shown by the fact that while the colonies on the plates from the original water indicated the presence of a considerable variety of bacterial forms, in the last plating from the cultures only one or two types remained.

The terrigenous bottom deposits of the Gulf of Maine contain varying amounts of organic matter originating partly from the organisms living in the sea bottom and sinking from the water above, but probably more largely from the organic matter present in a partly decomposed state in the sediments brought in from land. These forms of organic matter furnish the energy for the growth of the bacteria in the marine bottom. The total organic matter content in the mud and sand bottoms of the stations investigated is reported later, while a chemical study of the organic matter in sea bottoms or marine humus is reserved for another publication.

Table V

Presence of Anærobic Bacteria in the Marine Bottom

Medium		Solution				Agar			
Dilution		10		100		10		100	
Station No.	Depth of mud	1	2	1	2	1	2	1	2
1329	0–30 cm.	+	+	+	0	5*	2*	0*	2*
	30–60 cm.	+	0	+	0	12	2	4	8
	60–90 cm.	+	0	0	0	+	+	0	0
1330	0–30 cm.	+	+	+	0	30	18	10	8
1331	0–30 cm.	0	0	0	0	?	?	0	0

+ Indicates growth and gas formation.
* Figures = numbers of colonies in the agar shake-tube.

Mud samples for bacteriological investigation were obtained from Stations 1329 to 1332. The bottom at Station 1333 was made up of pebbles and no sample could be secured. The sand bottom of Stations 1334 to 1336 could not be sampled by the procedure outlined above because of the sand slipping from the tube as it was withdrawn from the bottom. However, at two of these stations sand samples were obtained by means of a Petersen grab. The numbers of bacteria found in these samples, both at the surface of the sample and at a depth of 10 cm., in the case of the mud bottoms, are given in Table IV.

TABLE VI

*Numbers of bacteria in sea water and in mud, as shown by the dilution method, using gelatin as the medium for bacterial development.**

Station	Depth †	Dilution cc.	Water	Mud
1329	Surface	0.1		++
1329	Surface	0.01		++
1329	Surface	0.001		++
1329	10	0.1		++
1329	10	0.001		+
1330	Surface	0.01		++
1330	Surface	0.001		+
1330	Surface	0.0001		0
1330	10	0.01		++
1330	10	0.001		0
1331	Surface	0.01		++
1331	Surface	0.001		++
1331	Surface	0.1	+	
1331	Surface	0.01	0	
1331	30	0.1	0	
1331	30	0.01	0	
1331	50	0.1	+	
1331	50	0.01	0	
1331	100	0.1	+	
1331	100	0.01	+	
1331	215	0.1	0	

* + indicates growth after 3 weeks incubation; ++ indicates very good growth.

† Depth in water, is, in meters, from surface of water; depth in mud is, in centimeters, from surface of mud.

The bacterial numbers in the surface layer of the mud were found to vary from 830 to 5400 per gram of dry mud. These figures for the mud may be compared with the values of 670 and 1280 per gram of dry sand at Stations 1335 and 1336. The two samples of mud (1329 and 1330) which contain more organic matter contain also more bacteria; variations which occur in the different mud samples may be ascribed to differences in organic matter content or to physical variations.

The total number of organisms decreases rapidly with the depth of mud. Thus at Station 1329, at a depth of 10 cm., the number of bacteria is only 1,720 as compared to 5,400 at the surface. The decrease is even more noticeable in the mud of Station 1332. The bacteria do not disappear rapidly, however, below the surface layer of the mud, but extend to considerable depths, as shown by the data for the presence of anærobic organisms (Table V). These bacteria were determined by the dilution method, using liquid medium kept under anærobic conditions, and by the shake-tube method, using agar medium. The results indicate the widespread occurrence of anærobic bacteria near the surface of the mud and their presence to depths as great as 90 cm. below the surface.

There is no doubt that the numbers of bacteria found in sea water and in the sea bottom by the plate method represent only a fraction of the total numbers of bacteria. This is due to the limitations of the method, whereby only a part of the bacterial population develops on the plate into colonies. In order to check this, several samples of water and mud were diluted and 1 cc. portions of the various dilutions added to sterile portions of 10 per cent gelatin in sea water. The results presented in Table VI show that whereas the plate method gave only 1 to 4 cells in 1 cc. of water from Station 1331, the dilution method with gelatin as a medium gave positive growth in some instances with 0.1 cc. of water and in some even with 0.01 cc. of water, indicating that at least 10 to 100 bacterial cells were present in 1 cc. of the water. In the case of the mud samples, abundant positive growth was obtained in most instances with 0.001 gram.

Results of bacterial investigation of the water and bottom samples of the Barents sea, recently reported by Butkewitch,[3] also bring out the fact that the dilution method may give 10 to 100 times as many bacteria as the plate method. The direct microscopic method gives even larger numbers, usually a thousand times greater than those obtained by the plate method. This difference is due to the fact that not only living but also dead bacteria are counted by the microscope, while many bacteria unable to develop on artificial media are seen with the microscope.

Occurrence of Specific Groups of Bacteria in the Sea

A number of bacteria active in certain specific processes, which are no doubt of considerable importance in the metabolism of the sea, were demonstrated both in the sea water and in the sea bottom. It is sufficient to mention here the presence of ærobic and anærobic nitrogen-fixing bacteria, of nitrifying bacteria, of agar-liquefying and cellulose-decomposing bacteria, of nitrate-reducing bacteria, of various spore-forming and non-spore-forming bacteria, and of a number of heterotrophic ærobic as well as anærobic bacteria. Some of these organisms will be discussed in detail later, while others need only be mentioned here in passing.

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[3] Butkewitch, W. C. Method of Bacteriological Investigation and Certain Data on the Distribution of Bacteria in the Water and Bottom of Barents Sea. Trans. Russian Oceanogr. Inst., 2: No. 2, 1932.

At least three types of cellulose-decomposing bacteria were demonstrated to be present in the sea. They belong largely to the groups described by Winogradsky as *Cytophaga, Cellvibrio,* and *Cellfalcicula.* They produce yellow and orange pigments or no pigment at all. They were found in the water from Station 1329 and to a less extent in the mud of the same station, but they were especially abundant in the tow water collected at Station 1336. This phenomenon may suggest the probability that these organisms, as well as the agar-liquefying bacteria, also present abundantly in the diatom tow, as pointed out previously, are not found to any great extent in the free water or in the mud, but develop in close connection with the growth of the plankton organisms, especially the phytoplankton; one would expect that the latter would offer a logical substrate for the growth of such bacteria. Some of these bacteria were able to attack both cellulose and agar, while others attacked cellulose alone and still others grew upon agar alone.

The agar-liquefying bacteria are represented in the sea by a number of different groups. They produce yellow, pink, or brownish pigments or no pigment at all. Most of them are ærobic, while some are anærobic. A detailed description of these organisms will be published in a later study. Chitin-decomposing bacteria have also been demonstrated in the sea water but these organisms have as yet been insufficiently studied.

It is commonly assumed that the sea harbors very few spore-forming bacteria. This may be true of the water itself; the sea bottom, however, especially the mud bottom, was found to harbor large numbers of such bacteria. The spore-forming bacteria could best be demonstrated in the nitrogen-free media inoculated with various dilutions of the mud samples. Many of the spores were found to have the typical appearance of *Clostridium,* so that one came to expect the development of these organisms in the nitrogen-free media inoculated with mud. This organism was accompanied by other bacteria, some of which were spore-forming. Many spore-forming bacteria were found abundantly in the calcium acetate-sodium nitrate medium used for the study of nitrate-reducing bacteria.

Anærobic bacteria were demonstrated by the use of liquid and solid media sealed with vaseline. Gas formation in the liquid media and colony development in the shake-tubes were taken as evidence of the development of these organisms. The ordinary peptone-glucose medium was used for this purpose. The cultures were inoculated with material from the mud profiles (Table V). The formation of colonies in the anærobic cultures brought out the fact that at least several hundred anærobic cells are present in each gram of moist mud, even to a depth of 60 cm. of mud.

Occurrence of Nitrogen-fixing Bacteria in the Gulf of Maine

The problem of nitrogen-fixation in the sea has always aroused considerable interest due to the importance of this phenomenon in marine metabolism. If it is true, as some oceanographers assume, that the sea produces as much plant and animal life as a similar area of land, the amount of nitrogen required for the synthesis of these numerous forms of life is quite enormous, since the nitrogen content of the various representatives of marine life varies from 0.7 per cent in the case of certain algæ, such as *Fucus,* to 10 per cent in the case of various animals which range from the lowly copepods to the large fish and mammals.

By the use of silica-gel plates and liquid media containing a layer of sand, the presence in the sea of the two important groups of non-symbiotic nitrogen-fixing bacteria, namely the aerobic *Azotobacter* and the anærobic *Clostridium,* was definitely demonstrated. The occurrence of organisms in the sea water and sea bottom of the Gulf of Maine, capable of growing on nitrogen-free liquid media, is brought out in Table VII. The nitrogen-fixing organisms were found both in the surface waters and in the marine bottom. Samples of water taken from Station 1331 at depths of 30, 100, and 215 meters gave largely negative results.

A detailed description of the methods used in this study as well as the nature of the organisms found and their nitrogen-fixing capacity will be reported in another publication. It suffices here to say that a nitrogen-free medium containing a carbon source (glucose, mannitol, salts of organic acids), a source of phosphate and organic iron was used. In the case of the plates, this medium was added to silica-gel prepared in large Petri dishes (15 cm. in diameter); it was then subjected to dialysis in tap water, and the plates finally soaked for a few minutes in boiled sea water. Each plate contained 2 grams of carbon source. The liquid medium was placed in large test tubes with a layer of sand on the bottom and sterilized.

The silica gel plate inoculated with mud from Station 1329 gave a

TABLE VII

Distribution of bacteria capable of growing on nitrogen-free media in ocean waters and marine bottoms

Station No.	Depth†	Dilution in cc. or grams	Sea Water						Marine Bottom					
			Mannitol			Glucose			Mannitol			Glucose		
			Growth*	Gas	Organisms‡	Growth	Gas	Organisms‡	Growth	Gas	Organisms‡	Growth	Gas	Organisms‡
1329	Surface	1.0	T, P	0	Bac.	P	0	Rods	—	—	—	—	—	—
1329	Surface	0.1	T	0	Bac.	T, P	0	—	T, P	+	Bac.	T	+	Bac.
1329	Surface	0.01	0	0	0	0	0	0	P	+	Bac.	T	—	—
1330	Surface	1.0	—	—	—	—	—	—	T, P	+	l.r.	—	+	sm.r.
1330	Surface	0.1	—	—	—	—	—	—	T	+	—	P, T	+	—
1331	Surface	1.0	T, P	—	sm.r.	T	0	sm.r.	—	—	—	—	—	—
1331	Surface	0.1	—	—	—				T	+	Bac.	T	+	Bac.
1331	Surface	0.01	—	—	—				T	+	Bac.	T	+	Bac.
1331	30	1.0	—	—	—	—	—	—						
1331	50	1.0	T, P	0	0	T	0	—						
1331	100	1.0	P	0	0	T	0	—						
1331	215	1.0	0	0	0	T	0	—						
1332	Surface	0.1	—	—	—	—	—	—	P	+	Bac.	T	+	l.r.
1332	10	0.1	—	—	—	—	—	—	—	—	—	T	+	—
1333	Surface	0.1	T, P	0	—	T, P	0	—	—	—	—			
1333	Deep	0.1	—	—	—	T, P	0	sm.r.						
1334	Surface	0.1	T	0	—	T	0	—	—	—	—			
1334	Deep water	0.1	T, P	0	sm.r.	—	—	—	—	—	—			
1335	Surface	1.0							T, P	+	sm.r.	T, P	+	l.r.
1336	Surface	1.0							P	—	sm.r.	P	+	sm.r.

* P = pellicle formation, T = turbidity.

† Depth in water, in meters, is from surface of water; depth in mud, in centimeters, is taken from surface of mud.

‡ Bac. = spore-forming rods; sm.r. = small rods; l.r. = large rods.

typical development of *Azotobacter chroococcum.* This organism was readily isolated and grown in pure culture. On nitrogen-free sea water media, with glucose or mannitol as a source of energy, it fixed four to six milligrams of nitrogen for one gram of carbohydrate in 21 days.

OCCURRENCE IN THE GULF OF MAINE OF BACTERIA CAPABLE OF OXIDIZING AMMONIUM SALTS TO NITRITES

If there is any one group of bacteria that has attracted the greatest attention of oceanographers and of bacteriologists alike, it is the group of nitrifying bacteria, or those organisms which produce nitrites from ammonium salts and nitrates from nitrites. A review of the various theories proposed to explain the occurrence of nitrite and nitrate in the ocean would in itself require considerable space. It suffices here to bring out only the results of the "Atlantis" expedition, to enable one to draw some very definite conclusions. It is important to emphasize in this connection that negative results do not necessarily mean the absence of the specific organisms but merely that the possibility exists that the medium used for demonstrating these organisms and the conditions essential for their development were not particularly favorable. Positive results are, however, much more important.

Of the two processes leading to the formation of nitrate, the first one, namely the oxidation of ammonium salts to nitrite, is usually considered to be more specific and more important, hence this process was more emphasized during this cruise.

Preliminary investigations of the water taken near shore brought out the fact that nitrite formation can take place in a sea water medium if proper conditions are provided. Three methods were used for this purpose: (1) Sand and nutrient salts—$CaCO_3$, $(NH_4)_2SO_4$, K_2HPO_4—were added, in proper concentration to flasks and these were sterilized; the fresh sea water taken from the various stations and at the different depths was introduced directly into these flasks. (2) In another set of flasks sand taken from Georges Bank was used in a fresh state and water taken from some of the stations was added. (3) In a third experiment, sea water, sand, and chemicals were placed in flasks, sterilized, and inoculated with mud from the various stations. The results obtained on the "Atlantis" cruise are presented in Table VIII. These results prove definitely that nitrite-forming bacteria are completely absent in the free sea water or are present there only in very limited numbers, while the sea bottom contains an abundance of such organisms. In the case of the mud, the presence of nitrifying bacteria was demonstrated at quite appreciable depths. This tends to demonstrate that nitrite formation and probably also nitrate formation take place in the sea bottom. The nitrite and nitrate ions will then diffuse into the water and will be gradually brought upwards by ocean currents, etc. Near the surface of the water, where active photosynthesis takes place, the nitrates tend to disappear due to the fact that they are rapidly consumed by the phytoplankton organisms.

OCCURRENCE IN THE SEA OF BACTERIA CAPABLE OF REDUCING NITRATES TO NITRITES AND TO ATMOSPHERIC NITROGEN

The presence in the sea of bacteria capable of reducing nitrates has attracted considerable attention from oceanographers, largely as a result of the hypothesis proposed by Brandt,[4] who attempted to base the whole system of marine metabolism on the occurrence and activities of these organisms. Preliminary studies in this laboratory have shown that when a liquid medium containing Ca-acetate, $NaNO_3$, and a layer of sand is inoculated with shore water or with mud, active nitrite formation will take place, accompanied by abundant gas evolution. When this medium was inoculated with mud, even in a dilution of 1:1000, the nitrate disappeared completely within 5 days. In other words, both the water and the mud close to the shore contain an abundance of organisms

TABLE VIII

Presence of nitrifying bacteria in sea water and sea bottom

Nitrite-forming bacteria in water			Nitrite bacteria in sea bottom		
Station No.	Depth of water	Nitrite formation in 20 days	Station No.	Depth of mud	Nitrite in 11 days
	meters			*cm.*	
1329	Surface	–	1329	0–30	+++
1329	Bottom	–	1329	30–60	++
1330	Bottom	–	1329	60–90	trace
1331	Surface	–	1330	0–30	++
1331	30	trace	1330	30–60	trace
1331	50	+	1330	60–90	trace
1331	100	–	1331	0–35	trace
1331	215	+	1336	Sand	+
1331	Bottom	–			
1332	Surface	+			
1332	50	+			
1332	100	+			
1333	Surface	trace			
1334	Surface	–			
1334	Bottom	trace			

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[4] Brandt, K. Ueber den Stoffwechsel im Meere. Wiss. Meeresunters. Kiel, N. F., **4**: 213–230, 1899; **6**: 23–79, 1902; **18**: 185–429, 1916–1920; **19**: 251–253, 1919.

capable of reducing nitrates to nitrites, as well as of reducing the latter completely to atmospheric nitrogen.

For the investigations carried out on the "Atlantis" cruise, a medium similar to the above and containing 10 grams Ca-acetate, 1 gram $NaNO_3$, 0.5 gram K_2HPO_4, and a trace of iron per liter of sea water was placed, in 25 cc. portions, in large test tubes containing a layer of sand. The medium was sterilized at 15 lbs. for 15 minutes. The tubes were inoculated immediately after the samples were taken, using various dilutions of water and mud. After two weeks incubation at air temperature, the cultures were examined microscopically and tested for nitrite (Tables IX, X).

TABLE IX

Relative abundance of nitrate-reducing bacteria in sea water

Station number	Depth of water	Dilution of water	Growth of bacteria	Microscopic examination	Gas evolution	Nitrite formation
	meters	*cc.*				
1329	Surface	1.0	Turbid	Large round cells and curved rods	–	+++++
1329	Surface	0.1	–	–	–	–
1329	Surface	0.01	–	–	–	–
1331	Surface	1.0	Turbid	Minute coccobacilli	–	+++++
1331	Surface	0.1	Slight turbidity	Minute coccobacilli	–	+++++
1331	Surface	0.01	–	–	–	–
1331	30	1.0	Turbid	Cocci and bacilli	–	+++++
1331	30	0.1	Turbid	Cocci and bacilli	–	+++++
1331	30	0.01	–	–	–	–
1331	50	1.0	Turbid	Cocci and bacilli	–	+++++
1331	50	0.1	–	–	–	–
1331	100	1.0	–	–	–	–
1331	100	0.1	–	–	–	–
1331	215	1.0	Slight turbidity	Long rods and cocci	–	+++++
1331	215	0.1	–	–	–	–
1333	Surface	0.1	Turbid	Small coccobacilli	–	+++++
1333	Surface	0.01	Turbid	Small coccobacilli	–	+++++
1333	Surface	0.001	Turbid	Small coccobacilli	–	+++++
1333	Bottom water	0.1	–	–	–	–
1333	Bottom water	0.01	–	–	–	–
1334	Surface water	0.1	–	–	–	–
1334	Surface water	0.01	–	–	–	–
1334	Deep water	0.1	–	–	–	+++++
1334	Deep water	0.01	–	–	–	–

Of all the cultures inoculated with water or with mud, only one gave positive gas evolution, namely, the one inoculated with 0.1 gram of mud from Station 1329. Positive growth of bacteria was always accompanied by a reduction of the nitrate to nitrite. This experiment tends to prove that the waters and the mud in the Gulf of Maine are able to reduce nitrates only to nitrites, but not to atmospheric nitrogen. In the case of the waters, nitrate reduction was active to a depth of 50 meters. Morphologically, the organisms developing in these cultures were found to differ considerably with the different inocula. Some were plain rods, others resembled *Azotobacter* cells, while still others were undeniably spores.

Table X

Relative abundance of nitrate-reducing bacteria in the mud

Station number	Dilution of mud	Growth of bacteria	Microscopic examination	Gas evolution	Nitrite formation
1329	0.1	Turbid	Spores	++	++
1329	0.01	Turbid	Few cells	–	+++++
1329	0.001	0	0	0	0
1330	0.1	Turbid	Oval rods, spores (?)	–	+++
1330	0.01	Turbid	Oval rods, spores (?)	–	++++
1330	0.001	Turbid	Oval rods, spores (?)	–	–
1330	0.0001	–	–	–	–
1331	0.1	Turbid	Spores	–	+++++
1331	0.01	Some turbidity	Spores	–	+++
1331	0.001	Some turbidity	–	–	+++
1331	0.0001	–	–	–	–
1332	0.1	Turbid	Spores, rods	–	+++++
1332	0.01	Turbid	Spores, rods	–	+++++
1332	0.001	–	–	–	–

Explanation of Nitrate Cycle in the Sea on the Basis of the Occurrence of Ammonia-oxidizing and Nitrate-reducing Bacteria

The results of the investigations carried out in connection with this expedition prove beyond any doubt that the marine bottom, both sand and mud, contains bacteria capable of oxidizing ammonia to nitrite, and later to nitrate; the process of nitrate formation in the sea must, therefore, be considered as bacteriological in nature. The results also show that the sea water and marine bottom contain bacteria capable of reducing nitrate to nitrite, in the presence of sufficient energy material, in the form of readily available carbon compounds. The fact that marine humus cannot be used as such a source of energy, while the amount of available carbohydrate in the mud is very limited, tends to emphasize that this process in the sea is only of questionable importance.

The problem finally remains to establish whether the limited occurrence of nitrite in sea water is due to the oxidation of the ammonia by nitrifying bacteria or to the reduction of nitrate by other bacteria. There is no doubt that the oxidation takes place chiefly at the bottom of the ocean; further that while the formation of nitrite from ammonium salts, under proper conditions of culture and with bottom material as an inoculum, can be readily demonstrated, nitrate formation takes place, under laboratory conditions at least, at a much later date. Reduction of the nitrate can be expected to take place both in the sea water and in the sea bottom; however, this process requires a supply of organic matter as an available source of energy. This can only be obtained through the photosynthetic activities of the planktonic organisms. It is no mere coincidence, therefore, that the maximum nitrite formation corresponds well with the maximum oxygen content in the water or the maximum photosynthetic activities.

The following hypothesis suggests itself at this particular point: Decomposition of the organic nitrogenous compounds takes place in the sea water but largely on the sea bottom, with the result that the ammonia is then liberated. This ammonia is rapidly oxidized by specific bacteria living in the bottom to nitrite and later to nitrate. This nitrate remains in the sea bottom and is not reduced, due to a lack of available energy for the nitrate-reducing bacteria and not to a lack of such bacteria. The small amounts of ammonia found in the sea water originate from the decomposition of the plant and animal residues in the plankton and in the water. The nitrate formed in the bottom gradually diffuses into the water, where it remains as such. On reaching the zone of photosynthetic activities, this nitrate is either consumed by the phytoplankton or is reduced by the nitrate-reducing bacteria to nitrite, which may also be gradually consumed by the plants. Very little nitrate reduction to gaseous nitrogen or complete denitrification is possible under normal sea conditions. Reduction of nitrate to nitrite does not mean necessarily any loss of nitrogen from the cycle of life in the sea.

Abundance of Marine Humus in the Bottom of the Gulf of Maine

A series of mud samples was taken with the small glass tube from the first four stations and from one sand station, in order to determine the abundance as well as the variability in distribution of the organic matter in the sea bottom. The methods of analysis, as well as the importance of these results in determining the origin and nature of the organic matter or the marine humus in the sea will be discussed in a later publication. The organic matter is reported in terms of carbon and nitrogen. In order to calculate the organic matter content of the mud or sand from the carbon data, the factor 1.724, which has found extensive application in soil investigations, may be used, for reasons discussed elsewhere.

TABLE XI

Distribution of marine humus in the sea bottom, as shown by the carbon and nitrogen content. Percentage of dry material.

Station No.	Sample No.	Carbon content	Nitrogen content	C/N
1329	1	2.46	0.284	8.7
1329	2	2.50	0.290	8.6
1329	3	2.48	0.290	8.5
1329	4	2.46	0.282	8.7
1329	5	2.37	0.278	8.6
1329	Average	2.45	0.285	8.6
1330	1	2.65	0.304	8.7
1330	2	2.66	0.284	9.4
1330	3	2.62	0.306	8.6
1330	4	2.70	0.294	9.2
1330	5	2.81	0.288	9.7
1330	Average	2.69	0.295	9.1
1331	1	1.58	0.156	10.1
1331	2	1.54	0.148	10.4
1331	3	1.55	0.144	10.8
1331	4	1.36	0.133	10.2
1331	5	1.29	0.133	9.7
1331	Average	1.46	0.143	10.2
1332	1	0.82	0.072	11.4
1332	2	1.20	0.096	12.5
1332	Average	1.01	0.084	12.0
1335	1	0.084	0.008	10.5
1336	1	0.12	0.009	13.3

The results reported in Table XI show that while the variation in the organic matter content is very small in some of the stations, it is quite appreciable in others. These results point definitely to the conclusion that determinations based upon a single sample may frequently give quite inaccurate results.

The distribution of marine humus in the mud profiles taken from the various stations is given in Table XII. A heavy brass sounding tube was used for this purpose. The depth of the mud brought up by means of this corer ranged from 75 to 115 cm. As soon as brought up, the cores were divided into three or four sections and later each analyzed separately. It is important to remember, of course, that the mud was compressed by the sampler and that the actual depth of the mud was greater than that shown by the figures. The analyses of the total carbon and nitrogen were made on the materials which had been dried in an oven at 100° C. There has been some question, however, as to the influence of drying upon the changes in the nitrogen content of the mud.

Table XII

Relative distribution of marine humus with the depth of the mud profile. On basis of dry material.

Station No.	Depth of mud	Carbon content	Nitrogen content	C/N
	cm.	*per cent*	*per cent*	
1329	0–30	2.58	0.290	8.7
1329	30–60	2.45	0.250	9.8
1329	60–90	2.32	0.238	9.75
1330	0–30	2.74	0.296	9.25
1330	30–60	2.75	0.254	10.8
1330	60–90	2.60	0.236	11.0
1331	0–35	1.58	0.140	11.3
1331	35–65	1.64	0.130	12.6
1331	65–90	1.61	0.128	12.6
1331	90–115	1.57	0.126	12.4
1331	90–115 (reddish mud)	1.04	0.080	13.0
1332	0–25	0.67	0.050	13.4
1332	25–50	1.12	0.092	12.2
1332	50–75	1.43	0.096	14.9

The samples obtained from Station 1329 were, therefore, analyzed for total nitrogen both in a moist and in a dry condition and both sets of results calculated on a dry basis (Table XIII). These results show that while in some instances there is no appreciable difference in the nitrogen content of the mud as a result of its preliminary drying in the oven, in other cases the difference obtained may be appreciable. All the analyses reported here were made on the oven-dried (60–90° C.) mud, due primarily to the ease of handling and to the greater uniformity of the samples.

Stations 1329, 1330, and 1331 show a gradual even if only a very limited decrease of the humus content with an increase in depth of mud, especially on the basis of the nitrogen figures. This is accompanied by a gradual decrease in the nitrogen content of the humus. This is due either to the greater decomposition of the nitrogen complexes with the age of the humus or to a difference in the nature of the materials deposited at different times. One is struck, however, by the great relative uniformity of the organic matter in the mud throughout the whole profile. One exception to this is found in one of the samples from the 90–115 cm. layer of Station 1331. This section of the profile was distinctly different in color from the rest of the profile and even from the duplicate part of another profile, being much redder in color. This proves again that even at one and the same station the mud may not be very uniform; this was brought out in Table XI for the data obtained from this station.

In the case of Station 1332, there is an increase in the humus content with an increase in the depth of the profile, as shown by both the carbon and nitrogen figures. This is due entirely to a difference in the nature of the material deposited at different periods, the surface material being much coarser than the lower layers. The amount of humus in the sand bottoms is very limited, usually equivalent to about 0.1 per cent carbon on the basis of the dry material. In view of the fact that no apparatus has been devised as yet to enable one to take a deep core of the sand bottoms, it is impossible to state how this humus content changes with the depth of the bottom. No information is available concerning any differences that may exist in the chemical nature of the humus in the mud and sand bottoms.

TABLE XIII

Influence of drying of marine mud upon its nitrogen content. On basis of dry mud.

Depth	Moist mud analyzed	Dry mud analyzed
cm.	*per cent N*	*per cent N*
0–30	0.306	0.290
30–60	0.282	0.250
60–90	0.239	0.238

On comparing the data of the humus content in the mud (Table XI) with those of the bacterial numbers (Table IV), one finds a certain definite correlation. This is especially true when one compares Stations 1329 and 1330 with a high humus content, on the one hand, with Stations 1331 and 1332 with a low humus content, on the other. One must keep in mind, of course, the fact that the numbers of bacteria were determined either in the very surface layer of the mud or in the 10 cm. layer, while the organic matter content was determined in the total core which ranged in depth from 15 to 25 cm. The bacteria are present most abundantly in the uppermost surface layer, where the organic matter is of recent origin and no doubt different chemically from the organic matter in the lower depths of the mud. The surface layer of the mud can be looked upon as the layer most active bacteriologically. The same is probably true of the sand, where the relative abundance of bacteria is considerably greater than the relative humus content. The abundance of humus can, therefore, be looked upon as only one factor controlling bacterial numbers and activities.

SUMMARY

A bacteriological survey has been made of the waters and bottom sediments in the Gulf of Maine and Georges Bank. The samples of water, plankton tow, and sedimentary material were taken under sterile conditions and subjected immediately to bacteriological analysis as soon as brought on board the "Atlantis."

The agar-plate method was used for the enumeration of the numbers of bacteria. This was supplemented to a limited extent by the dilution method. Various specific media were used to determine the distribution and relative abundance of certain groups of bacteria which are believed to take part in important marine processes.

The results obtained demonstrated the fact that the bacterial population of the sea can be divided into three groups on the basis of their habitat: (1) those forms which live in the sea bottom, especially in the surface layers; (2) those bacteria which live in the free water, this being possible only when the water contains in solution organic and inorganic substances which can serve as nutrients for the bacteria; (3) those bacteria which live largely upon or in association with the plankton organisms.

Sea water is a rather poor medium for the growth of bacteria, while the marine bottom is comparatively richer in the total number of bacteria capable of developing on the plate and in solution media. Mud bottoms contain more living bacterial cells than sand bottoms. However, the waters above the sandy bottom were found to contain many more bacteria than the waters above the mud bottoms. This may be due to the greater abundance of plankton organisms, especially diatoms, in the shallower seas with the sandy bottom, to the greater mixing of the waters, or to the greater absorption of bacterial cells by the mud bottom material than by the sand bottom.

The numbers of bacteria obtained by the plate method represent only a part of the bacterial population of the sea. This was shown by the fact that the dilution method, using gelatin as a medium for bacterial development, gave higher numbers than the plate method.

A decided parallelism was observed between the abundance of diatoms in the sea and abundance of bacteria. A comparatively large number of these bacteria were agar-liquefying organisms. In the artificial culture of diatoms, the numbers of bacteria were found to increase with the development of the diatoms. However, when the latter reached a maximum and began to die out, bacterial numbers did not diminish very rapidly; these bacteria seemed to be largely limited to a few specific types, the importance of which in marine processes still remains to be determined. These results seem to point definitely to the fact that the development of phytoplankton in the sea is accompanied closely by bacterial development. The bacteria feed upon the excretion products of the diatoms, algæ, and animal forms and probably upon these plankton forms themselves as soon as they die, thus bringing about their rapid disintegration and liberation of the nutrient elements in an available form.

Anærobic bacteria were found abundantly in the marine mud; these bacteria were present in the mud even at considerable depths. Their presence points to continued decomposition of the plant and animal debris of the ocean on and in the ocean bottom, even with an insufficient supply of oxygen.

The bacterial population of the sea was found to consist of a number of types, some of which take part in well-known processes which are of great importance in the metabolism of the sea, such as nitrogen-fixing, nitrite- and nitrate-forming, nitrate-reducing, cellulose-decomposing, agar-decomposing, chitin-decomposing, and many others. The importance of many common bacteria, both ærobic and anærobic, mostly non-spore-forming but also spore-forming, in marine processes still remains to be determined. The presence of nitrogen-fixing bacteria, comprising both the ærobic *Azotobacter* and the anærobic *Clostridium*, has been definitely demonstrated. One strain of the first group was isolated in pure culture and was found to be *Azotobacter chroococcum*, which fixed considerable quantities of nitrogen when grown on artificial culture media, with various carbon sources.

Bacteria capable of oxidizing ammonium salts to nitrites were found in the sea bottom, but only seldom in the sea water. For demonstrating the presence of these organisms in the sea, a medium containing a layer of sand and $CaCO_3$ and covered with a shallow layer of liquid containing an ammonium salt and the necessary minerals was used.

Bacteria capable of reducing nitrate were found abundantly both in the water, especially at the surface layers and the zone of photosynthetic activities, and in the sea bottom. However, these bacteria were able to reduce nitrate only to nitrite and not to nitrogen gas; only in one instance, namely, in the mud from the first station taken on this expedition, was there present an organism capable of bringing about the last process.

The cycle of nitrate in the sea is explained as follows: The nitrogenous constituents of the plant and animal residues in the sea are decomposed with the liberation of ammonia, largely in the sea bottom. The ammonia is oxidized in the sea bottom to nitrite and later to nitrate. The latter gradually diffuses into the waters. It is not reduced in the sea bottom or lower layers of water, largely because of a lack of available energy material necessary for the activities of the nitrate-reducing bacteria. In the zone of maximum photosynthetic activity such energy is available, hence nitrate-reduction may take place, but only to nitrite, since bacteria capable of reducing it to nitrogen gas are lacking under those conditions. The nitrite thus formed may also be assimilated by the phytoplankton and does not represent any loss of nitrogen in the sea.

The marine humus (total organic matter) content of the mud bottom in the Gulf of Maine is found to be more or less uniform in composition. This humus is best calculated from the total organic carbon. It was found that determinations based upon one sample may not give absolute evidence, since different samples may give considerable variation. The humus content usually decreases with the depth of the mud. In some stations, however, an increase in humus content with depth was observed. There is also a widening of the carbon-nitrogen ratio of the humus with an increase in depth. This points to a greater decomposition of the humus, especially of the nitrogenous complexes, with an increase in depth. An increase in humus content with depth of mud found in some stations merely points to the fact that the rate of deposition of the humus may vary considerably with different periods.

A definite parallelism was observed between the numbers of bacteria and abundance of humus in the mud.

A detailed review of the literature bearing upon the results presented in this report, as well as a detailed description of the methods used and a correlation of these results with the results of bacteriological investigations of the sea near shore, is reserved for a future publication.

Contribution No. 11

ON THE RESPIRATORY FUNCTION OF THE BLOOD OF THE PORPOISE

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ON THE RESPIRATORY FUNCTION OF THE BLOOD OF THE PORPOISE

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THE WOODS HOLE OCEANOGRAPHIC INSTITUTION [1]

The capture of two porpoises, *Phocæna phocæna* Linn., in the fish trap operated by the Marine Biological Laboratory has afforded an opportunity to obtain data which have not been available before on the conditions of equilibrium between the blood of a cetacean and the respiratory gases. The mammals of this order are so strikingly modified morphologically in adaptation to their aquatic life that an examination of the physico-chemical properties of the respiratory fluids is attended with some interest. While the results recorded in the present paper require confirmation and extension in many details, the general picture of the physico-chemical system involved in transporting the respiratory gases is sufficiently definite to warrant presentation in the present provisional form.

The porpoises were brought from the trap on May 14 and placed in a large floating crate at the laboratory. One died on the following day and was used to determine the proper approach for cardiac puncture. The other lived until May 18, when it was killed in the course of experiment. During this period it lay in the water rather quietly and did not struggle when held. On May 17 fifty cubic centimeters of blood was drawn by puncture of the left ventricle, the needle being inserted one inch behind and above the caudal edge of the left flipper. The porpoise did not struggle. A 10 cc. portion of this blood was preserved under oil, for the determination of the carbon dioxide content of arterial blood. The remainder was used for the determination of the shape of the oxygen and carbon dioxide dissociation curves. Coagulation was prevented by the use of dry sodium oxalate. The blood drawn at this time will be designated *Specimen A.*

The following day an unsuccessful attempt was made to measure the blood volume by the vital red method. Twenty cubic centimeters of blood were drawn from the heart with some difficulty and it appeared that both right and left ventricles had been pierced as a portion of the sample was not fully oxygenated. This sample was oxalated and used for the determination of oxygen capacity and cell volume and was designated *Specimen B.*

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[1] Contribution No. 4.

Immediately after drawing Specimen *B*, the porpoise went into asphyxial convulsions, evidently as the result of cardiac failure. The thorax was opened and 50 cc. of blood were drawn directly from the ventricle. This sample was designated as *Specimen C* and was used for determining the effect of hydrogen ion concentration upon the position of the oxygen dissociation curve.

METHODS

Oxygen and carbon dioxide contents were determined by using the Van Slyke constant volume analyzer. The gas mixtures were analyzed with a Haldane apparatus after equilibration and the pressures calculated by means of the equation developed by Bock, Field, and Adair (see Henderson, 1928, p. 384). The blood was equilibrated with the gas mixtures at 38°, preserved in blood-sampling tubes over mercury, and promptly analyzed. The original specimens of blood were preserved in the refrigerator until used, and all measurements were made within twenty-four hours of the time the specimens were secured.

TABLE I

Oxygen Capacity and Volume of Erythrocytes in the Blood of Aquatic Mammals

Species	Porpoise Phocæna phocæna			Dolphin Tursiops tursio	Sperm Whale Physeter macrocephalus	Sea Lion Eumetopias stelleri
Specimen	A	B	C			
Oxygen capacity, vol. per cent	22.15 22.20	19.8 19.7	20.44 20.34			19.8
Oxygen combined, vol. per cent	21.78	19.35	19.99	31.8	29.09	19.4
Volume of cells in 1 cc. blood, cc	0.35	0.35	0.36	0.517		0.29
Oxygen combined in 100 cc. cells, vol. per cent	62.2	55.4	55.6	61.5		67

THE OXYGEN CAPACITY OF THE BLOOD AND OF THE ERYTHROCYTES

Table I contains the data obtained from the three specimens of blood we have examined, together with results reported by Jolyet (1902) for the dolphin and sperm whale, and by Florkin and Redfield (1931) for the sea lion. The oxygen capacities we obtained, like those observed in the sea lion, are of the same magnitude as those commonly found in terrestrial mammals. They contrast strongly with the higher values recorded by Jolyet and Sellier (1896–97) for the porpoise (30.9 volumes per cent), by Jolyet (1902) for the dolphin and sperm whale, and with the values of 42.5 and 45.1 volumes per cent reported by Sudzuki (1924) for the blood of porpoises. Whether these differences are characteristic of the different species in question, or are due to accidental causes, such as a possible anæmic condition of the individuals yielding the lower values, or to experimental errors, cannot be decided until more data are available. We were impressed with the rapidity with which the corpuscles settled out when the porpoise blood was allowed to stand and believe that errors large enough to account for the differences reported for the different species might readily arise from this cause were it not guarded against. The observations are all in agreement in showing that a given volume of the corpuscles of these aquatic mammals combines more oxygen than does an equal volume of corpuscles of the terrestrial mammals, in which 100 cc. of corpuscles have an oxygen capacity of about 45 cc.

TABLE II

Data on carbon dioxide equilibrium in porpoise blood. Temperature, 38° C.

	Oxygen pressure	Carbon dioxide pressure	Carbon dioxide content
	mm. Hg	*mm. Hg*	*vol. per cent*
Specimen A	150 ca.	30.7	35.8
	150 ca.	51.3	46.0 46.2
Specimen C	150 ca.	41.0	25.6 25.5
	150 ca.	51.1	29.7 29.0
	1.4	51.8	32.45 32.30

THE CARBON DIOXIDE DISSOCIATION CURVE

Table II records the data obtained by equilibrating Specimens *A* and *C* with carbon dioxide. From these data the carbon dioxide dissociation curves illustrated in Fig. 1 have been constructed by making use of the approximation (Henderson, Bock, Dill, and Edwards, 1930):

$$\log T = A \log (p\mathrm{CO}_2) + B,$$

in which T is the total carbon dioxide content of the blood, A and B are constants, and pCO_2 is the carbon dioxide pressure. The curve constructed from data on Specimen A does not differ greatly from that of normal man, though the carbon dioxide combined at any pressure is somewhat less. The curve for Specimen C represents blood drawn following death from asphyxia, and the combined carbon dioxide is consequently less at each carbon dioxide pressure.

These curves may be used for comparing the buffer action of porpoise blood with that of human blood. Henderson, Bock, Dill, and Edwards (1930) have prepared a nomogram showing the relation between the slope of the carbon dioxide dissociation curve, its height, and the hemoglobin content of human blood based on an examination of some 117 specimens. In Table III are recorded the values of *Hb*, the hemoglobin content as measured by oxygen capacity; T_{40}, the carbon dioxide content at 40 mm. carbon dioxide pressure; and Δ, the difference in carbon dioxide content at 30 and 60 mm. carbon dioxide pressure as deduced from the curves and data describing Specimens A and C. In this table are also recorded the values of Δ characteristic of human blood having equivalent values for *Hb* and T_{40}, taken from the nomogram of these authors. Both specimens of porpoise blood have slightly higher values of Δ than does comparable human blood. The differences, however, are not greater than might be accounted for by experimental error. Comparable data for the blood of the sea lion, from the measurements of Florkin and Redfield (1931), and mean

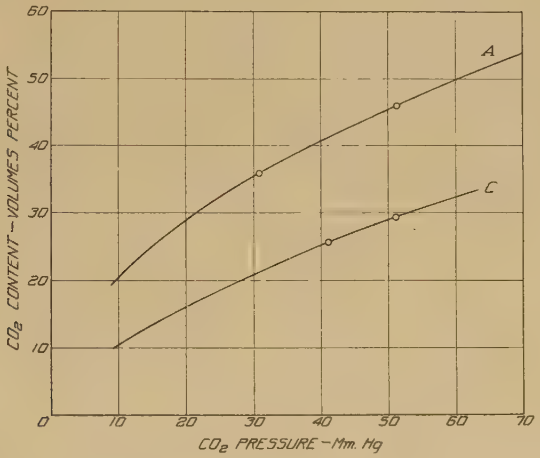

FIG. 1. Carbon dioxide curve of blood of porpoise. Upper curve, Specimen A. Lower curve, Specimen C. For data see Table II.

values for dog blood given by Dill, Edwards, Florkin, and Campbell (1932) are included in the table. The comparison serves to emphasize the similarity of the bloods when regarded as systems for transporting carbon dioxide.

Table II contains measurements of the carbon dioxide content of a sample of Specimen *C* equilibrated with carbon dioxide in the virtual absence of oxygen. Comparing the results with the value obtained from the curve in Fig. 1 for the same carbon dioxide pressure, it appears that the reduced blood combines about 2.86 volumes per cent more carbon dioxide than does oxygenated blood. This effect is similar to that observed in other mammalian bloods but is rather smaller than usual for blood of comparable oxygen capacity.

TABLE III

Comparison of Buffer Value of Oxygenated Porpoise and Sea Lion Blood with that of Man

Species	*Hb* Oxygen capacity	T_{40} CO_2 content at pCO_2 = 40 mm.	Δ observed	$T_{60} - T_{30}$ calculated from human blood	Ratio
	milliequiv. per liter	*milliequiv. per liter*	*milliequiv. per liter*	*milliequiv. per liter*	
Porpoise					
Specimen A............	9.73	18.18	6.52	5.80	1.12
Specimen C...........	8.93	11.22	5.12	4.70	1.09
Sea Lion..................	8.88	16.02	5.32	5.30	1.00
Dog (Mean)..............	9.7	18.1	5.78	5.76	1.00

THE OXYGEN EQUILIBRIUM

Data on the equilibrium of Specimen *A* with oxygen are presented in Table IV. In order to fit the measurements, the oxygen dissociation curve shown in Fig. 2 was drawn using Hill's equation

$$y = \frac{Kx^n}{1 + Kx^n} \times 100,$$

in which y is the percentage saturation of the hemoglobin, x the oxygen pressure, $K = 0.00102$ and $n = 2.1$. The carbon dioxide pressure of these samples was about 46.5, corresponding to a carbon dioxide content of 44 volumes per cent. Taking $pK = 6.14$, yields a value for pH of 7.29. The values for n which have been used to characterize the blood of man vary from 2.2 to 2.5. The shape of the oxygen dissociation curve of the porpoise so far as it is characterized by n does not differ markedly from human blood.

The portion of Specimen *A* which was collected under oil had a carbon dioxide content of 45 volumes per cent, corresponding to a carbon dioxide pressure of 49 mm. and a pH of 7.27. Consequently the oxygen dissociation curve in Fig. 2 represents approximately the conditions characteristic of the blood *in vivo*, if one neglects the change in oxygen equilibrium, due to the increased carbon dioxide content of the venous blood.

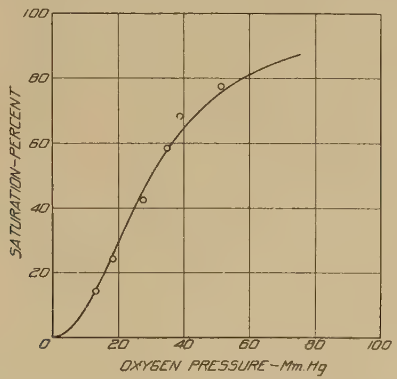

FIG. 2. Oxygen dissociation curve of blood of porpoise at a carbon dioxide pressure of approximately 46 mm.—equivalent to that of arterial blood. For data see Table IV.

THE EFFECT OF HYDROGEN ION CONCENTRATION ON THE OXYGEN DISSOCIATION CURVE

Table V contains data for the oxygen equilibrium of samples of Specimen *C* obtained at a variety of carbon dioxide pressures. Using

TABLE IV

Data on the equilibrium of oxygen with porpoise blood, Specimen A.
Temperature, 38° C.

Carbon dioxide pressure	Oxygen pressure	Oxygen content	Oxygen dissolved	Oxygen as oxyhemoglobin	Saturation
mm. Hg	*mm. Hg*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*
46.5	13.0	3.14	0.03	3.11	14.3
46.4	18.1	5.30	0.05	5.25	24.1
46.6	27.5	9.37	0.07	9.28	42.5
47.2	34.9	12.86	0.09	12.77	58.6
38.7	38.7	14.95	0.12	14.83	68.1
42.7	51.4	16.98	0.15	16.83	77.3
air	air	22.18	0.40	21.78	100.0

Hill's equation and taking the value of $n = 2.1$, the values of K, and of p_{50} (the oxygen tension at which the blood would be half saturated with oxygen) have been calculated. As an empirical procedure this practice involves only the assumption that the form of the oxygen dissociation curve, determined by the value of n, remains unchanged at various carbon dioxide tensions. This assumption is amply justified by the behavior of other mammalian hemoglobins. The hydrogen ion concentration of the various samples have also been calculated with the aid of the carbon dioxide dissociation curve for Specimen C in Fig. 1. From these values the relation between p_{50} and pH shown in Fig. 3 has been plotted.

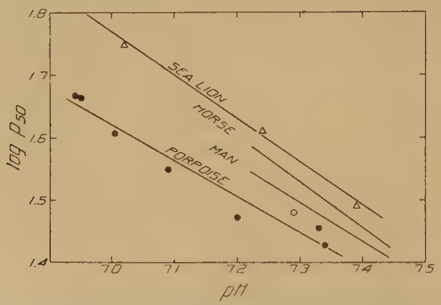

FIG. 3. Relation of p_{50}, the oxygen tension at which blood is half saturated, and the pH of the serum of whole blood. For data see Table V and references in text. Hollow circle, porpoise Specimen *A*. Solid circles, porpoise Specimen *C*.

For precise comparison of the properties of hemoglobin in the blood of different animals the properties should be referred to the pH of the cell contents. Unfortunately our studies did not include the measurements necessary to enable us to calculate this value. We have conse-

TABLE V

Data on the equilibrium of oxygen with porpoise blood (Specimen C) at different carbon dioxide tensions. Temperature, 38° C.

Carbon dioxide pressure	Oxygen pressure	Saturation	log p_{50} calc.	pH
mm. Hg	*mm. Hg*	*per cent*		
9.86	34.4	62.7	1.428	7.34
10.5	23.5	40.0	1.455	7.33
21.4	18.4	26.7	1.473	7.20
39.7	27.1	36.3	1.550	7.09
52.5	45.1	56.5	1.607	7.04
87.6	38.9	41.2	1.664	6.95
92.0	54.4	58.3	1.666	6.94

this species. My interpretations must be based on the fact that in my material no such body is present as that which he labels "motorium," quently compared the values of p_{50} obtained at different carbon dioxide pressures with those characterizing other bloods when the pH of the serum of the whole blood was the same. This comparison is included in Fig. 3, the data for the horse and for man being taken from the nomograms given by Henderson (1928); that for the sea lion is calculated from the data of Florkin and Redfield (1931). When compared on this basis the hemoglobin of the porpoise does not differ strikingly from that of other mammals and does not differ from the blood of normal man much more than the blood of men differ from one another.

Discussion

The preceding observations all tend to indicate that the general physico-chemical properties of the blood of the porpoise are very similar to those of the terrestrial mammals. Even in the strictly quantitative aspects of the various properties of the blood one can detect very little variation from the mean conditions observed in human blood. The increased concentration of hemoglobin in the corpuscles of the cetaceans and the sea lion is perhaps the only clear-cut condition which might be regarded as an adaptation to the more rigorous respiratory requirements of aquatic life.

Unfortunately, our attempts to determine the blood volume of the porpoise were unsuccessful. This relation is, in our opinion, the most important aspect of the respiratory mechanism which remains to be examined. One cannot help being struck by the richness of the muscular tissue in hemoglobin. It seems probable that in this condition, and perhaps in other modifications of the chemical situation in the muscles, adaptations to aquatic life may be found.

It should be pointed out that the porpoise is not in the habit of remaining submerged for long periods of time when compared to the larger Cetacea and certain other aquatic mammals. Parker (1932) records that in a porpoise confined in a large tank, the average respiratory interval was 15.48 seconds. The shortest interval observed was 6.5 seconds, the longest 31.7 seconds. These are not strikingly greater than the intervals of inspiration in man and might easily be maintained, at least temporarily, by voluntary effort in his case. When feeding in the open sea, one might expect the intervals to be greater and Jolyet and Kükenthal (quoted by Parker) note that dolphins may remain under water much longer, Jolyet setting the maximum at about fifteen minutes. The general testimony of whalers indicates that the larger Cetacea are in the habit of remaining submerged for much longer periods. Whether more distinct adaptations of the physico-chemical mechanisms of the blood will be found in such forms remains to be seen.

Summary

The properties of the blood of the porpoise, *Phocæna phocæna*, considered as a system for the transport of oxygen and carbon dioxide, have been examined.

It does not appear that the morphological changes adapting these animals for aquatic life are accompanied by significant modifications in the physico-chemical properties of the blood.

The only striking characteristic of porpoise blood, when compared with that of terrestrial mammals, is an increased concentration of hemoglobin in the corpuscles.

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Contribution No. 12

It has been amply demonstrated (18), in the study of the decomposition of plant residues on land, that some of the organic constituents, like the sugars, cellulose, and certain hemicelluloses (40–44 per cent carbon), decompose rapidly; whereas others, like the lignins (62–64 per cent), decompose slowly; and still others, like the proteins (48–52 per cent carbon), may actually increase in total concentration, as a result of the synthesis of new proteins by microorganisms. As a result of this specific type of decomposition, there is a marked change in the carbon content of the residual humus as compared with that of the original plant residues. Probably similar transformations take place in the decomposition of plant and animal residues in the sea, no doubt of a different order of magnitude, due to a difference in the chemical nature of the residues, in the conditions under which decomposition is taking place and in the microörganisms which are active in the decomposition processes.

Certain relationships between the two most important elements of the marine humus, namely, the carbon and nitrogen, point to a similarity in chemical nature to land humus. Boysen-Jensen (2) found that apparently pure sand of the North Sea contained, on a dry basis, 0.34 per cent carbon and 0.027 per cent nitrogen, with a carbon-nitrogen ratio of 12.6. The carbon content of the marine bottom material from the various parts of the Limfjord varied from 0.58 to 4.3 per cent, with a C:N ratio of 10.6 to 12.4. The clay bottom of the Kattegat, at depths of 30 to 35 m., contained 2.3 per cent carbon, with a C:N of 11.5, while the mud of the inner part of the Roskilde Fjord contained as much as 10.2 per cent carbon. At greater ocean depths, namely, at 800 to 2,200 meters, Boysen-Jensen, using eight samples obtained from the Ingolf expedition, found 0.3 to 1.6 per cent carbon, with a C:N of 10.2. One may conclude from these results and those of Petersen and Boysen-Jensen (7) that the organic matter content of the sea bottom is very variable; however, the carbon-nitrogen ratio of the organic matter is more or less constant, varying from 8.1 to 12.6, with an average of 11.1. If one assumes that the carbon content of the marine humus is 50 per cent, the nitrogen content will thus be found to be about 5 per cent.

Moore (4) found that the organic matter content of marine mud, as measured by the total nitrogen, changes with depth of the deposit. In the case of the muds and sandy muds of the Clyde Sea area, the nitrogen content varied from 0.089 to 0.379 per cent at a depth of 0 to 5 cm., from 0.083 to 0.330 per cent at 5–10 cm., from 0.074 to 0.295 per cent at 10–15 cm., and from 0.074 to 0.302 per cent at 15–20 cm. depth. The decrease with depth is gradual, but not very appreciable. In a few instances there was an increase in the nitrogen content with the depth of mud. No correlation was observed, however, between the depth of the water and the nitrogen content of the mud.

In a series of determinations of the organic carbon content of marine sediments, Trask (15, 16) reported a range from 0.2 per cent, for a sand bottom, to 4.2 per cent, for a clay bottom, with an average of 1.8 for 25 near-shore sediments and 2.1 per cent for Channel Island deposits (California). The organic carbon content of sediments of past geologic ages was found to be similar to that of recent sediments. The C:N ratio of a number of representative sediments from all over the world was 8.4 ±1.0, with a range of 5.3 to 13.3. Recent marine deposits contain more nitrogen in respect to carbon than do land soils and lacustrine deposits. The C:N ratio of deposits of past geologic ages was found to be greater than that of recent deposits. Trask concluded that the organic matter content of the sea bottom is influenced by the bottom configuration; because of its greater ease of transportation, it increases in concentration as the texture becomes finer. Deposits in depressions and in closed basins contain more organic matter than do those on adjoining ridges and slopes. Trask also found a rapid drop in the organic matter content of the marine sediments with depth, the drop becoming less with an increase in depth; he emphasized the fact, however, that since this depends on the rate of deposition, the organic matter content may be sometimes greater at lower depths than at the surface.

The very surface layer of the mud or of other marine bottom material is rich in detritus, namely, plant and animal residues in the process of decomposition, and in the so-called jelly-like material (8). This detritus contains an abundant bacterial population (21) and is different in nature from the true marine humus or the organic matter which has already undergone extensive decomposition and has become an integral part of the bottom material. Murray and Hjort (6) suggested that the organic substance of the marine deposits serves as a fertilizer for the surface layers of the marine bottom. Petersen (7) also believed that the mud-eating animals obtain nutrients from the surface layers of the mud. It still remains to be determined, however, to what extent true marine humus can offer nutrient material to animal life.

The few available determinations on the abundance and chemical nature of organic matter in the bottom of deep seas are based almost entirely on the loss-on-ignition method. In the case of the Globigerina ooze, figures ranging from 0.8–6.0 per cent have been found, the Challenger expedition reporting 3.6 to 10.4 per cent; red clay was found to give 2.7 per cent, by the loss on ignition method, Radiolaria ooze — 4.3 to 7.4 per cent, and mud of Diatomaceae — 4.0 per cent (1).

As to the chemical nature of the marine humus, the few investigations of Boysen-Jensen, in which he tried to establish the fact that Zostera contributes largely to the formation of this humus, and of Trask and Hammar, who found a similarity in chemical composition between the soil humus and marine humus, practically exhaust all the available information.

The following experiments represent an attempt to study the abundance of humus in the marine bottoms, its nature and possible origin, and its further decomposition.

EXPERIMENTAL

Abundance of humus in the marine bottom, especially in marine muds

The humus content of the marine bottom was calculated from the amount of organic carbon found. This carbon was determined by the use of Tiurin's (14) modification of Schollenberger's method. The actual amount of dry material taken for analysis does not play any important part in the determination, as shown by the results reported in table 1, on four different types of marine mud with varying humus content and one sample of humus isolated from the mud by treatment with dilute alkali solution and precipitation with acid. Several of the mud samples, as well as certain humus preparations isolated from land soils, were analyzed for total carbon both by the regular combustion method, using an electrically heated furnace and absorbing the CO_2 liberated, and by the simplified oxidation procedure. The results obtained by the two methods checked up very well.

The nitrogen determinations were made by the usual Kjeldahl method using the air-dry bottom material; it was found later (20), however, that in some instances some of the nitrogen may be lost in the process of drying of the material, especially in the case of surface layers of mud or recent formations. In some cases, the loss on ignition was also determined using the common procedure of igniting the oven-dry material to constant weight.

The following investigations were based largely upon the study of the humus content of mud collected from Buzzard's Bay, from Cape Cod Bay, and from several stations in the Gulf of Maine. The samples of mud were obtained by three different methods: (*a*) by the use of a small glass cover similar to the one described by Moore and Neill (5), (*b*) by means of a heavy brass pipe, used extensively at the Oceanographic Institution, (*c*) by means of a Petersen grab. The organic matter content of marine sand bottoms was studied only to a limited extent. A few samples of bottom material were made available from the deeper layers of the sea, through the Wilkins expedition and a cruise of the "Atlantis" to Bermuda.

The mud samples from the aforementioned stations belong to the group usually described as "blue muds," formed by terrestrial or semi-pelagic sedimentation. The color of the mud is due to the organic matter and the FeS content. Frequently the large profiles of mud from the Gulf of Maine, a meter or so in depth, showed a marked change in color with a change in depth. The samples of mud were allowed to dry, usually at temperatures not above 85–90°C. The results of analysis are based upon the total dry mud, not freed from the sea salt.

TABLE 1

Carbon content of marine mud, as determined by the simplified oxidation procedure

NATURE OF MUD SAMPLE NUMBER	AMOUNT OF DRY SAMPLE USED*	TITRATION RESULTS	EQUIVALENT TITRE	CARBON CONTENT
	mgm.	*cc.*	*cc.*	*per cent*
0	0	30.2	0	0
1	300	20.3	9.9	1.98
1	500	13.9	16.3	1.96
2	300	21.7	8.5	1.70
2	500	15.5	14.7	1.76
3	465	18.4	11.8	1.52
3	382	20.5	9.7	1.52
4	539	25.9	4.3	0.48
4	661	25.3	4.9	0.45
α-humus	20	13.4	16.8	50.4
α-humus	50	4.5	40.8	49.2

* Ten cc. of the chromic acid solution was used for all the mud samples and 15 cc. for the two humus samples.

It was essential to determine first how uniformly the humus is distributed in marine sediments, not only vertically but also horizontally. A series of samples of mud were taken from three stations in the Gulf of Maine; the water at those stations was 190 to 225 m. deep; the location of these stations and the results of the bacteriological investigation of the water, as well as of the mud itself, are reported in detail elsewhere (20). Several samples of mud were taken from each station, by means of the small glass corer, as close to one another as the movement of the boat made possible; these samples represent only the surface 10 to 20 cm. of mud. The results reported in table 2 show that, in general, there is very little variation in the horizontal distribution of the humus in the mud, when the samples are not taken too far apart as shown by the carbon and nitrogen content, as well as by the carbon-nitrogen ratio.

However, at greater distances, even only a few miles, the samples of mud may vary considerably in humus content, as shown in tables 3 and 4, where the humus content of the muds in the vicinity of Woods Hole are reported. The method of determination of humus by loss on ignition gives results which are considerably in excess over the total organic matter, as calculated from the total carbon and nitrogen; there is, however, a very definite parallelism between the two sets of data. Those samples of mud (sample 1 and surface portion of sample 5) which give the highest loss on ignition also contain the largest amount of carbon and nitrogen.

TABLE 2

Distribution of marine humus in sea mud, as shown by the carbon and nitrogen content

Per cent of dry material

STATION NUMBER	SAMPLE NUMBER	CARBON CONTENT	NITROGEN CONTENT	C/N
1329	1	2.46	0.284	8.7
1329	2	2.50	0.290	8.6
1329	3	2.48	0.290	8.5
1329	4	2.46	0.282	8.7
1329	5	2.37	0.278	8.6
1329	Average	2.45	0.285	8.6
1330	1	2.65	0.304	8.7
1330	2	2.66	0.284	9.4
1330	3	2.62	0.306	8.6
1330	4	2.70	0.294	9.2
1330	5	2.81	0.288	9.7
1330	Average	2.69	0.295	9.1
1331	1	1.58	0.156	10.1
1331	2	1.54	0.148	10.4
1331	3	1.55	0.144	10.8
1331	4	1.36	0.133	10.2
1331	5	1.29	0.133	9.7
1331	Average	1.46	0.143	10.2

When samples are taken from the same location, at different periods, varying results may be obtained (table 4). A series of samples of mud were collected from Cape Cod Bay, at three different intervals. Some of the samples were taken within the bay itself, between Race Point and end of the canal, and some near the Cape Cod Lightship, at definite distances from shore, station 5 being nearest to the shore (10 miles) and station 8 farthest from the shore (25 miles). Considerable variability was found in the humus content of the mud taken presumably from the same station, but at different periods. The reason for

TABLE 3

Humus content of marine muds in Buzzard's Bay

NUMBER	LOCATION, NEW BEDFORD HARBOR	MOISTURE IN FRESH MUD	ON PER CENT BASIS OF DRY MATERIAL			C/N
			Loss on ignition	Carbon	Nitrogen	
		per cent				
1	Above harbor	54.6	7.45	2.30	0.226	10.2
2	Center of harbor	49.4	5.90	1.96	0.188	10.4
3	Below harbor	39.0	5.25	1.76	0.164	10.7
4	Sandy part of harbor	23.0	1.85	0.58	0.047	12.4
5 Upper 4 cm.	Near sewer outlet	55.0	7.65	3.37	0.250	13.5
5 Lower 4 cm.	Near sewer outlet	24.3	3.50	1.21	0.090	13.4

TABLE 4

Humus content of marine mud in Cape Cod Bay and near Cape Cod Lightship

STATION NUMBER	DATE OF SAMPLING	CAPE COD BAY			
		Moisture content of fresh mud	Humus in dry material		
			Carbon	Nitrogen	C/N
		per cent	*per cent*	*per cent*	
1	VIII/24/32	29.6	0.82	0.078	10.5
2	VIII/24/32	....	2.98	0.306	9.7
3	VIII/24/32	44.0	1.84	0.180	10.2
4	VIII/24/32	53.6	2.51	0.206	12.2
6	VIII/24/32	67.4	3.04	0.266	11.4
4	IX/20/32	52.5	2.36	0.226	10.5
6	IX/20/32	64.4	3.92	0.325	12.1
		CAPE COD LIGHTSHIP			
6	VII/13/32	58.8	1.42	0.147	9.7
7	VII/13/32	53.3	1.90	0.206	9.2
5	VIII/24/32	25.0	0.34	0.042	8.1
7	VIII/24/32	54.8	1.55	0.160	9.7
8	VIII/24/32	56.6	1.73	0.206	8.4
6	IX/20/32	53.9	1.99	0.166	12.0
7	IX/20/32	63.4	2.22	0.236	9.4
8	IX/20/32	66.3	2.32	0.257	9.0

this variability is to be looked for primarily in the following two causes: (*a*) the non-uniformity of the sea bottom and (*b*) the fact that the organic matter content of the surface of the mud varies from that of the lower layers and that the surface layer is apt to be changeable, because of water movements and depositions of fresh material. This is especially true of such near-shore stations as these represent. The carbon-nitrogen ratio of the humus in the muds from Buzzard's Bay was found to range from 10.2 to 13.5 and in the humus of muds from Cape Cod Bay, from 8.1 to 12.2. The latter variation is almost exactly the same as that reported by Petersen.

Schokalsky (9) measured the stratification of the muds at the bottom of the Black Sea. He suggested that the alternating layers found there are probably a result of a winter terrigenous deposit (gray) and a spring and summer planktonic deposit (black). This phenomenon enabled him to calculate that it takes 50 years to deposit a layer of mud 1 cm. in thickness. Similar stratification was reported by Moore (5) for the mud in the Clyde Sea area.

TABLE 5

Abundance of marine humus in muds deposited at different ages

STATION NUMBER	DEPTH OF MUD IN PROFILE	MOISTURE CONTENT OF FRESH MUD	ON PER CENT BASIS OF DRY MATERIAL		C/N
			Carbon	Nitrogen	
	cm.	*per cent*			
1329	0–30	65.0	2.52	0.290	8.7
1329	30–60	60.8	2.45	0.250	9.8
1329	60–90	58.8	2.32	0.238	9.8
1330	0–30	59.9	2.74	0.296	9.3
1330	30–60	58.2	2.75	0.254	10.8
1330	60–90	57.4	2.60	0.236	11.0
1331	0–35	50.0	1.59	0.148	10.7
1331	0–35	49.5	1.56	0.132	11.8
1331	35–65	46.9	1.64	0.130	12.6
1331	65–90	48.8	1.61	0.128	12.6
1331	90–115	46.9	1.57	0.126	12.4
1331	90–115 (reddish mud)	46.8	1.04	0.080	13.0
1332	0–25	31.2	0.67	0.050	13.4
1332	25–50	36.7	1.12	0.092	12.2
1332	50–75	37.7	1.43	0.096	14.9

In order to determine whether there is a marked difference in the humus content of the mud deposited at different ages, several profiles were obtained, by the use of the large brass borer, from the Gulf of Maine (20). The length of these cores when brought up to the surface ranged from 75 to 112 cm.; they were probably deeper in the natural state, since they have undergone some compression by the sampler. The cores were divided into three or four sections and, as soon as brought to the laboratory, dried and analyzed. The humus content of the mud taken from the different sections varies considerably from station to station, as shown in table 5. The variation with depth is more uniform. In the case of the first three profiles (stations 1329–1331), there is a gradual but slow reduction in the humus content with an increase in the depth of mud; however, in the fourth profile (station 1332), there was an increase in humus content with depth. In the case of the last profile, the inorganic particles of the surface layers of mud were decidedly coarser and became finer with an increase in depth. This was not the case of the other three profiles. The mechanical analyses of the mud profiles from station 1329 (fig. 1) and 1332 (fig. 2) were made by Mr. Stetson of the Oceanographic Institution (3), to whom the author wishes to express his sincere indebtedness.

The carbon-nitrogen ratio of the humus was found to increase gradually with an increase in the depth of mud; this is probably because under the anaerobic conditions prevailing in the mud an increase in depth results in a greater, even if only very slow, decomposition of the nitrogenous than of the non-nitrogenous constituents.

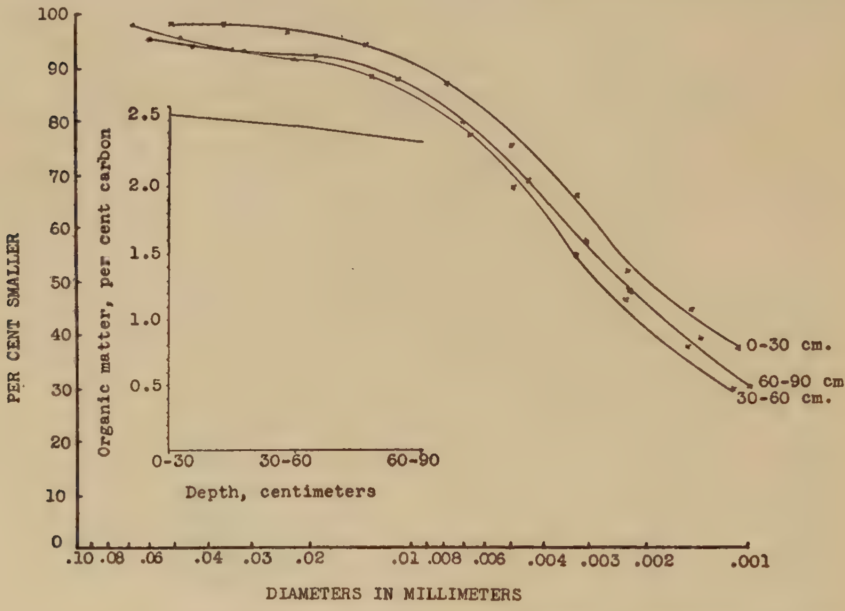

Fig. 1. Change in Mechanical Composition, as Determined by Hydrometer Method, and Humus Content, as Measured by Organic Carbon, with Depth of Marine Mud.—Station 1329

Only a few samples of bottom material obtained at greater distances from shore were available for determinations of the humus content. Table 6 shows the results of analyses of a mud profile brought by Sir Wilkins from the Nautilus expedition. This profile was taken north of Spitzbergen at 81–52 Lat. N. and 13.40 Long. E., at a depth of 1,620 m. The length of the core, in the compacted form, was 42 cm. The total organic matter content of this mud was lower than that of the mud in the Gulf of Maine; there was also a gradual decrease in humus content with an increase in depth of the mud, as shown both by the carbon and nitrogen data. It is of interest to note particularly that the carbon-nitrogen ratio of the humus in this mud as well was 10.65 to 12.44. Table 7 shows the abundance of humus at great depths of water, truly oceanic in nature. These samples of bottom material were taken during the "Atlantis" cruise to the Bermudas in September, 1932. The humus content of the sediment is rather low, ranging from 0.60 to 0.78 per cent, calculated on the basis of the total organic carbon (using the factor 2.0). The loss on ignition of these samples was very high; in the case of station 1359, a loss of 20.2 per cent

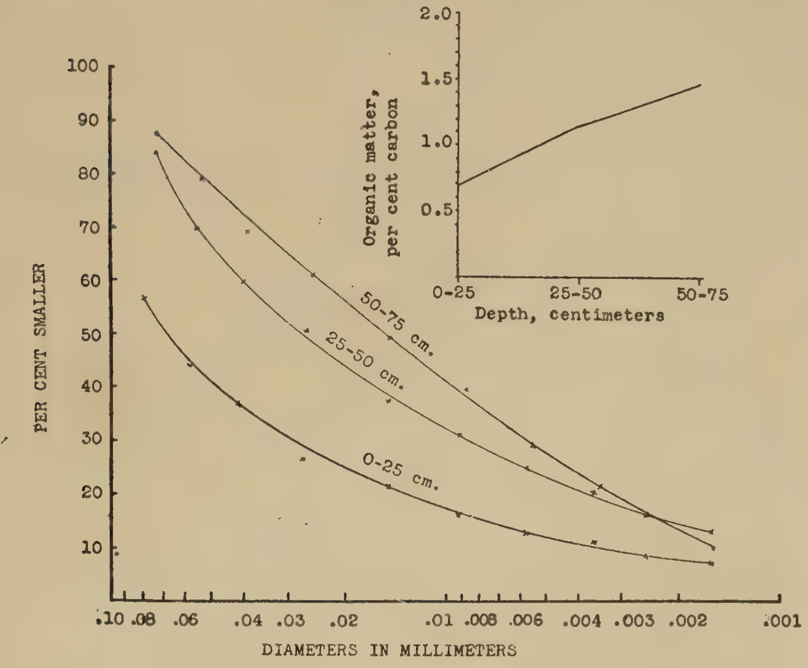

FIG. 2. CHANGE IN MECHANICAL COMPOSITION, AS DETERMINED BY HYDROMETER METHOD, AND HUMUS CONTENT, AS MEASURED BY ORGANIC CARBON, WITH DEPTH OF MARINE MUD.—STATION 1332

TABLE 6

Marine humus in mud from north of Spitzbergen

On basis of dry material

DEPTH OF MUD	ORGANIC CARBON	TOTAL NITROGEN	C/N
cm.	*per cent*	*per cent*	
0–14	0.852	0.080	10.65
15–28	0.672	0.054	12.44
29–42	0.468	0.044	11.10

was obtained on the basis of the dry material, as contrasted with 0.746 per cent as calculated from the organic carbon.

The humus content of sandy bottoms is considerably lower than that of muds and even of deep sea bottoms. Two samples of sand bottom were taken (20) at George's Bank (samples 1335 and 1336); the water at those stations was 74 and 64 meters deep, respectively. The organic carbon of the two samples of sand bottom was found to be 0.084 and 0.120 per cent and the nitrogen 0.008 and 0.009 per cent, respectively; the C:N ratio of the two samples was, therefore, 10.5 and 13.3.

The results of the quantitative analyses of the humus content in marine bottoms permit the drawing of the following conclusions: (*a*) The organic matter content of marine bottoms varies considerably, even at a distance of a few miles from one another and frequently in close proximity, as shown both by the organic carbon and total nitrogen content; (*b*) in most instances, there is a slow and gradual diminution of the amount of humus present in the mud with an increase in depth, although in some instances the reverse may hold true; (*c*) the carbon-nitrogen ratio of the humus in the mud is more or less constant, namely, about 10:1; the tendency is for this ratio to be narrower in muds containing a high content of humus and wider in muds with a low humus content; this ratio was found to range between 7.5:1 and 14.9:1; (*d*) the humus content of sand bottom and deep sea deposits is considerably less than that of mud bottoms; (*e*) a definite relation seems to exist, to a certain extent at least, between the size of the inorganic particles in the sediment and the amount of humus, the latter increasing with a decrease in size of the former.

TABLE 7

Humus content of the true oceanic bottom

STATION NUMBER	SAMPLE NUMBER	DEPTH OF BOTTOM	CARBON CONTENT	NITROGEN CONTENT	C/N
		m.	*per cent*	*per cent*	
1348, lat. 39°36′ N long. 64°52′ W	B.T. 2	4,800	0.348	0.042	8.3
1351, lat. 39°37′ N long. 63°50′ W	B.T. 3	5,040	0.432	0.043	10.0
1359, lat. 32°12′ N long. 64°36′ W	B.T. 5	2,720	0.373	0.032	11.7
1363, lat. 33°39′ N long. 67°28′ W	B.T. 6	5,400	0.456	0.061	7.5

Chemical nature of marine humus

Our knowledge of the chemical nature of marine humus is very limited. Still less is known concerning the mechanism of its formation, and there is no information whatsoever which would enable us to state whether the organic matter in marine muds is of the same chemical nature as that of sand bottoms and deep oceanic deposits or not. The fact that the organic matter of marine sediments forms only a small portion of a system predominantly inorganic in nature, the fact that it is formed from plant and animal residues, and through the agency of microörganisms, and further its specific physical (color) and chemical properties (carbon content, nitrogen content, and C:N ratio) would justify a comparison of the organic matter in marine bottoms, or marine humus, with that of the organic matter in land soils or soil humus. However, differences between the chemical composition of the residues of land plants and marine plants, as well as differences in the nature of the microörganisms active in the decomposition processes in land soils (fungi, actinomyces, bacteria, and animals) and in sea water and marine bottoms (bacteria and animals only) would lead one to expect certain differences in the chemical nature of this humus. In the case of soil formations, the humus of mineral soils varies considerably from that of forest soils and especially from that of peat bogs; one may also expect that the humus of mud bottoms should vary in chemical nature from that of sand bottoms and deep ocean sediments, as a result of difference in origin and decomposition processes.

In the study of the chemical nature of humus in land soils, chemists have assumed that this humus consists of a few specific "humic acids"; some went as far as to claim that humus is comparatively simple in chemical composition. However, as a result of the investigations of Schreiner and Shorey (11) at the Bureau of Soils in Washington, it was shown that numerous compounds can be isolated from the various so-called "humic acid" fractions of the soil humus. The writer and his associates have also shown (18, 19) that although the humus of land soils contains small amounts of fatty and waxy substances, of carbohydrates, especially hemicelluloses, and of other organic compounds, it consists chiefly of two complexes, namely, proteins and lignins. The last two groups make up nearly 75 per cent of the total humus of the soil.

On treatment of soil with dilute alkali solutions, a large part of the total humus, frequently 70 to 75 per cent, can be dissolved. When this solution is acidified with mineral acid, a heavy precipitate is obtained which is insoluble in acid. This fraction represents only a part of the humus brought into solution by the treatment with dilute alkalies. When the acid filtrate is adjusted to neutrality by means of an alkali, another precipitate is obtained, which is largely inorganic in nature. The first precipitate was designated by the writer (17) as "α-humus;" it is commonly known in the literature as "humic acid" or "humic fraction." The second fraction was designated as "β-humus"; this is frequently considered as a part of the "non-humic fraction" of the humus. The α-humus fraction of the soil humus contains only about 1 to 3 per cent ash, 55–58 per cent carbon and 2.5–3.5 per cent nitrogen; the β-humus fraction consists of 65–80 per cent of inorganic material (largely aluminum silicate) and of a smaller part of organic matter.

A procedure similar to the one outlined in the foregoing was used for the study of the humus in marine muds, in an attempt to elucidate the chemical nature of this humus and compare it with that of humus in field soils. A quantity of mud was collected in Buzzard's Bay, near Wepecket Island, using the Petersen grab. The dry mud analyzed as follows:

	per cent
Loss on ignition	7.18
Total organic carbon	2.01
Carbonate carbon	0.15
Total nitrogen	0.23
Carbon-nitrogen ratio	8.72

Four-hundred-gram portions of the air-dry mud were extracted with 400-cc. portions of a 4 per cent NaOH solution, for 1 hour, at 15 pounds pressure. The extracts were filtered through paper and the residues again extracted with additional quantities of 200 cc. of the alkali solution for 30 minutes at 15 pounds pressure, and again filtered. The residues after the second extraction were washed twice with distilled water. The combined filtrates and washings were neutralized in the cold with a 1:1 hydrochloric acid solution; an excess of the acid, equivalent to about one-half of the amount used for neutralization, was then added. A heavy, dark-colored precipitate was produced; it was removed by filtration through paper, washed with distilled water until free from acid, and dried. This precipitate may be designated as the "α-fraction," to make it comparable to the similar fraction obtained by the same procedure from land soils. The acid filtrate from the α-fraction was now neutralized to litmus with 40 per cent NaOH solution, to a pH of about 4.8. A heavy precipitate was again formed, which was filtered off, washed, and dried; this fraction formed a light colored powder and was designated as β_1. The filtrate from the β_1 fraction was now treated with concentrated $CaCl_2$ solution, which resulted in another heavy precipitate; this fraction was also removed by filtration, washed, dried, and designated as the "β_2;" it was also very light in color. The filtrate from the β_2 fraction was treated with two volumes of 95 per cent ethyl alcohol. A heavy precipitate was formed, which was allowed to settle, and was removed by centrifuging. It was washed with 95 per cent alcohol and acetone and dried over sulfuric acid. A light-brown colored preparation was thus obtained, which was designated as the "γ-fraction" of the marine humus.

The composition of the four fractions prepared from 400 gm. of air-dry marine mud is given in table 8, and the total nitrogen and carbon balances are given in tables 9 and 10. The extraction of the mud with 4 per cent alkali solution, at 120°C., removed 84 per cent of the total organic matter, or humus in the mud, as measured by the nitrogenous constituents, and only 70 per cent of the humus, on the basis of the relative amount of organic carbon extracted. Nearly a half of this humus, as measured by the carbon content, was recovered in the α-fraction, or the acid precipitate of the alkali extract. A large part of the nitrogen of the organic matter in the mud is left, however, in the filtrate.

TABLE 8

*Yield and chemical composition of the four fractions obtained from marine mud by alkali extraction**

Per cent of dry material

FRACTION	α	β_1	β_2	γ
Total yield, *gm*	5.081	8.997	10.789	3.280
Ash in dry material, *per cent*	4.9	89.4†	75.5†	52.1
Organic carbon in dry material, *per cent*	54.5	3.36	4.99	22.1
Nitrogen in dry material, *per cent*	4.32	0.36	0.53	2.46

* From 400 gm. of dry mud.

† The high carbonate content of these two fractions, especially of β_2, accounts for the discrepancy between the loss on ignition and the organic carbon content.

TABLE 9

Nitrogen balance in 400 gm. of dry marine mud

	mgm.
Total nitrogen in mud used for extraction	920
Nitrogen in residual mud, not extracted by the alkali solution	148
Nitrogen extracted by 4 per cent NaOH solution	772
Nitrogen accounted for, α-fraction	219.5
β_1-fraction	32.4
β_2-fraction	57.2
γ-fraction	80.7
Nitrogen in final filtrate	271.3
Total nitrogen accounted for	661.1
Nitrogen not accounted for (largely lost as NH_3 in the process of extraction with hot alkali solution)	110.9

TABLE 10

Organic carbon balance in 400 gm. of dry marine mud

	mgm.
Total organic carbon in mud	8,010
Organic carbon in residual mud, after NaOH extraction	2,412
Carbon extracted by 4 per cent NaOH solution	5,598
Carbon accounted for in α-fraction	2,769
β_1-fraction	302
β_2-fraction	539
γ-fraction	725
Total carbon accounted for	4,335
Carbon not accounted for in 4 fractions and present largely in the filtrate from the γ-fraction	1,263

By subtracting the ash from the total dry materials of the α-fraction and assuming that all the nitrogen is in the form of protein, with 16 per cent nitrogen and 50 per cent carbon, one can divide the organic matter of this fraction into two complexes; namely, the protein complex, which makes up 27 per cent of the total α-fraction, and another complex making up the remaining 68.1 per cent of the fraction which is non-nitrogenous and which contains 60.2 per cent carbon; this high carbon content is characteristic of lignin complexes, as shown later. One can conclude, therefore, that the α-fraction is largely a ligno-protein complex. The β_1 and β_2 fractions contain only a small amount of organic matter and can be left out of further consideration.

The organic matter of the γ-fraction contains 46.2 per cent carbon and 5.14 per cent nitrogen. By assuming that the nitrogen is in the form of protein or its derivatives and by allowing 16 per cent nitrogen and 50 per cent carbon for the protein, one finds that the non-nitrogenous part of the organic matter of the γ-fraction contains 43.6 per cent carbon. This would place this fraction among the carbohydrates. On treating some of the dry γ-fraction with a hot dilute mineral acid, a considerable amount of reducing substance was formed. By boiling it with 12 per cent hydrochloric acid and collecting the CO_2 in standard $Ba(OH)_2$ solution, then titrating back with standard oxalic acid solution, a large amount of uronic acid anhydride could be demonstrated. The uronic acid content of the original mud was 0.219 per cent, and that of the γ-fraction was 4.1 per cent. A large part of the uronic acid present originally in the mud was thus found in the γ-fraction. Some of the uronic acid was destroyed on treatment of the mud with the hot alkali solution. The presence of uronic acid complexes in marine sediments can easily be understood, since polyuronides form important constituents of many of the marine algae (21).

A detailed analysis of the proximate chemical composition of the α and γ fractions, isolated from marine mud by the procedures outlined, is given in tables 11 and 12. These results show that the α-fraction is largely a ligno-protein complex, the total lignin and protein accounting for 85.90 per cent of the total organic matter in this fraction. On treatment of the α-fraction with 80 per cent sulfuric acid for 2 hours in the cold, then diluting with 15 volumes of water and boiling for 5 hours, 78.8 per cent of the total α-fraction was left unhydrolized. The remaining 14.1 per cent of the organic substance in the α-fraction is made up of certain carbohydrates, which give on hydrolysis with acids reducing sugars and non-reducing compounds; this is further confirmed by their carbon content. The α-fraction obtained from marine mud is thus found to be very similar in chemical composition to the same fraction obtained from field and garden soils, with the exception that it is considerably higher in protein; the corresponding fraction from soil usually contains 3.0 to 3.5 per cent nitrogen, against 4.54 per cent in the fraction from marine mud. The higher relative nitrogen content of the mud and of its α-fraction, as compared with field soils, is probably due to the fact that a much larger part of the organic matter in marine mud is formed from animal residues, which are higher in protein, than is the case of field soils.

The chemical nature of the γ-fraction is distinctly different from that of the α-fraction. It represents largely a protein-carbohydrate complex, with a high uronic acid content. There is no doubt that the uronic acid compounds of this fraction were derived from the plant residues (algae, diatoms) in the ocean, which have resisted decomposition by bacteria, or from the slimy material synthesized by bacteria and algae. The fact that the humus of field

TABLE 11

Proximate chemical composition of α-fraction of marine humus

	DRY, ASH-FREE MATERIAL
	per cent
Total carbon	57.30
Total nitrogen	4.54
Protein equivalent of nitrogen	28.38
On treatment with cold 80 per cent sulfuric acid; sugar, as glucose	5.26
Ligno-protein fraction (unacted upon by 80 per cent sulfuric acid)	78.80
Protein content of "lignin fraction"	21.28
"Lignin" content of α-fraction	57.52
"Lignin" content of organic matter of α-fraction	60.55
Carbon as protein in α-fraction (on basis of 50 per cent carbon)	14.19
Carbon as hemicellulose yielding sugar	2.10
Carbon as lignin (64 per cent carbon)	36.81
Total carbon accounted for	53.10
Carbon not accounted for (57.30 − 53.10)	4.20
Total organic matter not accounted for [100 − (28.38 + 5.26 × 0.9 + 57.52)]	9.4
Carbon content of organic fraction not accounted for, and dissolved by boiling dilute acid (4.20 × 100:9.4)	44.7

TABLE 12

Proximate chemical composition of γ-fraction of marine humus

	DRY ASH-FREE MATERIAL
	per cent
Total carbon	46.20
Total nitrogen	5.14
Protein equivalent	32.1
Total uronic acid anhydride	8.50
On treatment with cold 80 per cent sulfuric acid, fraction was completely dissolved, except 3.5 per cent, which was practically all ash; reducing sugar formed, as glucose	21.80

soils and peats contains a high amount of uronic acid complexes has been pointed out elsewhere (19); the presence of uronic acid in soil humus has actually been demonstrated by Shorey (12). The large amount of reducing sugar obtained on hydrolysis of the γ-fraction points to the presence of other carbohydrates, besides the uronide in this fraction.

Among the various specific chemical complexes of marine humus, the pentosans received special consideration. Boysen-Jensen (2) reported that pentosan makes up 0.27 to 1.06 per cent of the total organic matter, the pentosan carbon comprising 5.2 to 8.7 per cent of the total carbon. Recent studies (12, 19) on the soil organic matter brought out the fact that the complex formerly known as pentosan and determined by the furfuraldehyde method, is largely a uronic acid compound. In light of these investigations, one may suggest that the "pentosan" of Boysen-Jensen is probably a carbohydrate derived to a large extent from the uronic acid complexes in the marine humus.

These analyses, even if based only upon the humus of one sample of marine mud, permit one to conclude that this humus has a specific chemical composition. The humus constituents can be classified into three groups: (*a*) lignins and their derivatives, (*b*) proteins, and (*c*) carbohydrates, largely polysaccharides and polyuronides. One can also demonstrate the presence of a small amount of fatty and waxy substances, as well as other chemical constituents in lower concentrations. There is no doubt that the humus in sand bottoms and especially in the deep ocean sediments may vary in chemical composition from that of muds, and possibly that the humus in other marine muds may vary from that of the mud used for this study, but this is just as one would expect it.

Factor for calculating the humus content of sea bottom sediments

It was pointed out previously that the use of the factor 2.0 for calculating the total organic matter or humus content in marine bottoms from the amount of organic carbon found is open to criticism, because insufficient attention is paid to the processes of humus formation and to its chemical nature. However, one would hardly be justified in adopting the factor 1.724 commonly used for the calculation of humus in land soils, without further investigation, because of the marked difference in chemical composition between marine plants and land plants; animal residues and excreta contribute of course to both formations. There is no doubt that marine humus in muds which are not very far distant from shore is partly of terrestrial origin, as a result of which one can expect to find in the humus a fraction of a ligno-protein nature (α-fraction) with a high carbon content.[3] However, since the residues of marine plants which consist largely of carbohydrates with a low carbon content also contribute to the marine humus, one would expect that these would tend to lower the carbon content of the humus. The isolation of the specific fractions described in the foregoing may enable one to make certain calculations which will tend to throw a definite amount of light upon this problem.

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[3] The decomposition of the Zostera plants growing close to shore will no doubt also contribute a similar complex.

The treatment of the mud with 4 per cent NaOH solution resulted in the removal of 70 per cent of the organic matter from the mud, as calculated from the carbon content. Of this organic matter, just one-half, or 35 per cent of the total carbon, was recovered in the α-fraction. The lignin made up 60 per cent of this fraction; in other words, 21 per cent of the total organic matter in the humus soluble in the hot NaOH solution, under pressure, is in the form of lignin-like complexes. The total protein content of that part of the humus which was soluble in the alkali solution, as calculated from the amount of nitrogen extracted, makes up 30.2 per cent of the total humus, assuming a carbon content of 50 per cent. The remaining 18.8 per cent of the dissolved organic matter is made up partly of carbohydrates (hemicelluloses) and related compounds and partly of various other complexes the exact chemical nature of which is not known. The carbon content of the humus extracted with 4 per cent NaOH solution can therefore be calculated as follows:

COMPLEX	PER CENT OF TOTAL HUMUS	THEORETICAL CARBON CONTENT	CARBON FRACTION BROUGHT INTO SOLUTION
		per cent	
Lignin	21.0	64	13.44
Protein	30.2	50	15.10
Hemicelluloses and others	18.8	45	8.46
Total extracted	70.0	....	37.00

These results enable one to compute tentatively that the percentage of carbon in the humus extracted by 4 per cent NaOH solution is about 53. Since this extracted part represents 70 per cent of total humus extracted from the mud, one may conclude from these results that the carbon content of marine humus is about 53 per cent; the factor to be used for calculating the humus content in marine bottoms on the basis of the organic carbon content would, therefore be 1.887. One assumes thereby that the carbon distribution in the 30 per cent of the humus in the mud not extracted by the alkali solution is the same as in the extracted part. That this is not fully the case is brought out by the fact that a much smaller amount of nitrogen than of carbon is left in the residual mud, when compared with the original mud. Whether the residual carbon and nitrogen represent, partly at least, chitinous materials, or other carbohydrate-like complexes, still remains to be determined. Certainly their carbon content should not be higher than is the case of the complexes dissolved by the hot alkali treatment.

An attempt was made to analyze the marine humus also by another procedure developed for the study of soil humus (22). One-hundred-gram portions of the dry mud were extracted for 8 hours with sulfuric ether in Soxhlets.

The ether-soluble portion was determined by evaporating the ether and drying the residue in a weighing bottle. The residual mud was then treated with boiling 95 per cent alcohol, for 10 hours; the alcohol extract was evaporated on a water bath, the residue dried, weighed, and ignited. This was followed by extraction with hot water, on a boiling water bath, for 2 hours, then by boiling for 2 hours with 2 per cent HCl solution. The residue left after the last treatment was washed, dried, and weighed; aliquot portions were then treated in the cold with 80 per cent H_2SO_4, for 2 hours, followed by dilution with 15 volumes of water and boiling under 15 pounds pressure for 30 minutes; the residue was washed, dried, weighed, and analyzed for total carbon and nitrogen. The results of the analyses are given in table 13. The carbon content of the various fractions was calculated, assuming a certain carbon content which is characteristic of the major constituents of the fraction. The theoretical carbon content of the marine humus, as calculated by this system of analysis was 52.8 per cent. One is thus able to account for 5,125 mgm. of the organic matter out of a total of 5,320 mgm. actually present in the mud, by assuming that the carbon content of the humus in the mud (2.82 per cent total carbon) is 53 per cent.

TABLE 13

Chemical composition of humus in marine mud

100 gm. of dry mud containing 2.82 per cent carbon

ORGANIC MATTER FRACTION	YIELD	APPROXIMATE CARBON CONTENT	TOTAL CARBON
	mgm.	*per cent*	*mgm.*
Ether-soluble	57	70	40
Alcohol-soluble organic matter	592	50	296
Hot water-soluble organic matter	389	46	179
Organic matter soluble in 2 per cent HCl*	527*	42	222
Soluble in cold 80 per cent H_2SO_4	919†	42	386
Protein insoluble in cold 80 per cent H_2SO_4	769	50	385
Lignin-like complexes (insoluble in cold 80 per cent H_2SO_4)	1,872	64	1,198
Total	5,125	.....	2,706
Carbon content of organic matter, per cent	.....	.....	52.8

* Calculated from organic carbon in solution.

† Calculated by loss in carbon content on treatment.

The two sets of analyses give a close enough factor for determinations of this type, which involve organic complexes the exact chemical nature of which is not yet understood. One can thus conclude that the carbon content of the humus in marine mud is 53 per cent and that a factor of 1.887 can be recommended for calculating the organic matter of the mud on the basis of its organic carbon content. This factor may, of course, be found to be too low in the case of the humus in deep oceanic sediments and too high in case of older geological deposits.

Origin of marine humus

Any study of the chemical nature of marine humus must also consider, (*a*) the origin of this humus, which results from the decomposition of plant and animal residues in the water and in the marine bottoms, by the action of bacteria, preceded or not by animal digestion, in addition of course, to terrigenous deposition; (*b*) the further decomposition or mineralization of the marine humus, with the result that the important elements carbon, nitrogen, and phosphorus are returned to circulation in the sea, in the form of carbon dioxide, ammonia, and phosphoric acid, and are thus made available again for the growth and photosynthetic activities of the phytoplankton.

The problem of decomposition of marine algae and of zooplankton by bacteria is discussed in detail in another publication (21). Only a small part of the organic matter built up by marine plants passes through the animal body, and the larger part is destroyed through direct bacterial action when the plants die (13). It remains to be determined whether these dead plants are decomposed as a whole, leaving no residues, or whether substances are formed in the process of decomposition which are similar to the humus of land soils. According to Boysen-Jensen (2), Zostera is the chief source of organic matter in the sea bottom, especially in the fjords, and not the plankton; in the deeper and more open waters, however, the plankton organisms were found to be an important source of the organic matter in the sea bottom. The high nitrogen content of the sea bottom suggested the idea that the non-nitrogenous constituents rather than the nitrogenous complexes have undergone decomposition. The latter were believed to be transformed into humic compounds which are less readily destroyed. Jensen actually demonstrated that the older the Zostera plant, the less in the amount of nitrogen which can be made readily soluble by the action of pancreatic enzymes upon it.

The presence of different complexes in the marine humus, especially the α-fraction, which is a ligno-protein complex, and the γ-fraction, which is a carbohydrate-protein complex, points to the possibility that this humus, at least in the case of deposits not far from shore, may be derived from different sources. There is no doubt that the decomposition of Zostera, which contains lignin and cellulose similar to land plants, may lead to the formation of ligno-protein complexes. The fact remains, however, that few other marine plants contain very much lignin. The possibility thus suggests itself that the ligno-protein complexes (α-fraction) found in marine humus are partly of terrestrial origin and may also partly be due to the decomposition of plant remains at shore points; however, other constituents of the marine humus, as in the case of the γ-fraction, may chiefly be of marine origin.

Decomposition of marine humus

There is no doubt that marine humus, like land humus, is resistant to further rapid decomposition by bacteria; otherwise it would not accumulate very extensively in the marine sediments. The possibility exists, however, that this humus is not absolutely resistant, but undergoes a slow but gradual decomposition. To test this, 150-gm. portions of dry marine mud were placed in long-necked flasks, moistened with 50 cc. of tap water or salt water, and connected with an aeration apparatus; CO_2-free air was passed over the mud, then through $N/6$ $Ba(OH)_2$ solution, in order to remove the CO_2 liberated in the process of decomposition. The solution was then titrated with $N/6$ oxalic acid. The muds were incubated at 28°C. for 14 to 25 days. At the end of that period, the mud was removed from the flasks and analyzed for ammonia and nitrate. The moist mud was plated out on suitable media; only bacteria were found, but not fungi or actinomyces, a phenomenon characteristic of the marine mud under natural conditions. For comparison, a similar amount of field soil, known as Sassafrass loam, with a similar amount of organic matter, was allowed to decompose under the same conditions. The results are given in table 14.

TABLE 14

Decomposition of the humus in marine mud, as compared with that of normal soil

MATERIAL USED	150 GM. OF DRY MUD—50 CC. WATER					
	Water used	Incubation	CO_2 liberated	Nitrogen liberated		C/N of products liberated
				NH_3-N	NO_3-N	
		days	*mgm.*	*mgm.*	*mgm.*	
Marine mud	Tap water	25	75.3	13.5	0	5.6
Marine mud	Salt water	14	55.8	11.1	0	5.05
Field soil	Tap water	25	43.7	0.5	4.8	8.2

These results show that the organic matter in the marine mud, which has previously been dried, is able to decompose very actively, more so even than a similar amount of organic matter in soil, as shown by the greater evolution of CO_2. There was a much greater relative liberation of the nitrogen in an available form from the mud than from the soil, as shown by the narrower C:N ratio of these two important elements, liberated in a mineralized state. This may be due partly to the relatively greater nitrogen content of the mud, as well as to the fact that the nitrogenous complexes in the mud humus can be decomposed more rapidly than those of the soil humus. The last assumption is born out by the fact that the carbon-nitrogen ratio of marine humus usually widens with an increase in depth of mud in sediment, as shown in table 5. In the case of land soils, the tendency is for this ratio to become narrower with depth of soil. In the flasks containing the land soil, the ammonia formed in the decomposition of the organic matter by the microorganisms was rapidly changed to nitrate, as a result of the activity of the nitrifying bacteria. In the case of the marine mud, however, all the nitrogen liberated was left as ammonia, because of the fact that no nitrification took place in the mud, under the particular experimental conditions. It is possible that the drying of the mud resulted in the destruction of such organisms.

These results serve to emphasize the fact that the humus in marine mud, when placed under favorable conditions, can decompose readily and liberate the nutrient elements back into circulation. Actually, 150 gm. of mud containing 3,000 mgm. of carbon liberated 55.8 mgm. of carbon as CO_2 in 14 days and 75.3 mgm. in 25 days, under two sets of conditions, while, out of a total of 345 mgm. of nitrogen, 11.1 mgm. and 13.5 mgm. were liberated as ammonia in the corresponding periods. There is no doubt that the drying of the mud was partly, at least, responsible for this comparatively rapid decomposition of the humus; however, even the organic matter in moist mud was found later to decompose but at a slower rate.

Importance of humus in marine processes

It has been suggested (20) that some of the most important processes carried out by bacteria in the sea take place in the sea bottom, whether the latter is made up of mud, sand, or the various marine oozes; these activities take place probably in the very surface layers of the bottom. Among these, one need only mention the decomposition of the numerous residues of plant and animal life in the sea, which sooner or later reach the bottom, and the formation of nitrates, by oxidation of the ammonia first to nitrite and then to nitrate. The relatively greater abundance of bacteria in the sea bottom, especially in the very surface layers, as compared with the numbers in the sea water, is sufficient evidence to the importance of the marine bottom as a seat of bacterial activities in the sea. The abundance of humus in the marine bottom seems to have a very definite influence upon the bacterial numbers; one finds frequently a very close correlation between the abundance of humus and the abundance of bacteria in marine muds, as shown elsewhere (20): the greater the humus content of the mud the greater were the numbers of bacteria found to be within a certain close area of the sea. One need not expect, however, that this correlation will always hold. The physical conditions of the sea bottom, especially the mechanical composition of the inorganic fraction, the colloid content, etc., may influence considerably the abundance of bacteria.

The marine humus thus makes the sea bottom a favorable medium and substrate in which the bacteria grow and upon which they live. The rôle of the marine humus in animal nutrition is still a matter of conjecture; however, there is no doubt that it makes the sea bottom also a more favorable medium for the growth of numerous animals that live in it or upon it.

In addition to the aforementioned function, humus also plays an important part in the sedimentation processes taking place in the sea. It is sufficient to refer here to the preliminary investigations of Raymond and Stetson (8), on the possible significance of a jelly-like organic material on the surface of the sea bottom, as an agent of transportation of sand grains.

SUMMARY

A study has been made of the abundance and distribution of organic matter in the marine bottom, largely mud sediments. A few sand bottoms and deep oceanic deposits were also analyzed.

It is suggested that the organic matter in the marine bottom be designated "marine humus," to make it comparable to the soil humus, found in land soils.

The abundance of humus in the sea bottom was found to vary considerably, depending on the distance from shore, the topography of the sea bottom, the nature of the inorganic residues, and the depth of the water.

The humus content in the marine mud was found to diminish gradually with depth of mud; in some cases, however, the humus content may increase with depth, depending on the nature of the material deposited.

Marine humus is characterized by a more or less constant ratio of carbon to nitrogen, which ranges from 8 to 12; the lower the humus content the wider the ratio.

Marine humus is shown to be very complex in chemical composition; it can be separated into a number of fractions which possess distinctly different properties. Several such fractions were isolated, one of which, termed "α-fraction," consists predominantly of lignin and protein and is similar to a corresponding fraction isolated from land soil, and another, termed "γ-fraction," is largely a hemicellulose-protein complex.

It is suggested that the abundance of organic matter, or marine humus, in the sea bottom be measured by determining the total organic carbon by some convenient method and calculating the humus by the use of the factor 1.887.

Humus is formed in the marine bottom as a result of bacterial decomposition of plant and animal residues of the sea. Close to shore it is partly of terrestrial and partly of marine origin. Marine humus is more resistant to further decomposition than are fresh plant and animal residues, especially at lower depths. In the presence of sufficient oxygen, the humus undergoes a process of slow decomposition, as shown by the liberation of a continuous stream of CO_2 and ammonia.

Humus gives certain characteristic properties to the marine bottom, making it a more favorable medium for the growth of bacteria and animal forms.

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Contribution No. 13

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THE EVOLUTION OF THE RESPIRATORY FUNCTION OF THE BLOOD

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ACCORDING to current doctrines the mammals are the product of an evolutionary development which is traced back through the lower classes of vertebrates and is usually considered to have had its origin among the invertebrate phyla. In recent years the respiratory function of mammalian blood has been studied in great detail. Considered as a physico-chemical system concerned with the transport of oxygen and carbon dioxide, its characteristics are now well known and in many ways satisfactorily understood (35). Recent publications make it possible to compare these properties of the blood in at least one or more representatives of the various classes of lower vertebrates and of certain invertebrates. One can consequently inquire what changes in the characteristics of the blood parallel the supposed evolutionary development.

In considering the evolution of the vertebrates certain general tendencies are apparent which have an obvious relation to the respiratory function of the blood. In the first place, the change from an aquatic to a terrestrial habit of life involves a profound alteration in the method of aerating the blood and in the conditions to which the blood is exposed in the respiratory organs. In the second place, the general tendency throughout the development has led to organisms increasingly capable of intense activity. This has required the development of more effective arrangements for the circulation of the blood and the transport of gases by it in order to care for greater metabolic requirements. One of the factors which has favored the greater activity of which the birds and mammals are capable is the development of a constant, high body temperature which has at the same time fixed rather definitely one of the conditions under which the respiratory function of the blood takes place.

The transport of oxygen by vertebrate blood is due practically entirely to the hemoglobin present in it. Consequently the conditions under which oxygen is carried depend upon the specific properties of this substance. The transport of carbon dioxide, on the other hand, while depending in large part on reactions in which hemoglobin is involved, is a less specific phenomenon, since other proteins such as serum albumen and globulin and certain inorganic substances play a considerable rôle. The transport of carbon dioxide is consequently dependent in its details upon the general processes whereby the kidney and other organs regulate the composition of the blood.

The hemoglobins are substances which may be decomposed into hematin and a protein, globin. The former compound contains iron in complex combination with protoporphyrin. It is to the hematin fraction that the transport of oxygen by hemoglobin is due, oxygen being combined with hemoglobin in strict proportion to its iron content. The hematins derived from the hemoglobin of different species appear to be identical, since it has been shown that the same porphyrin may be derived from the hematin of mammals, fish, and the insect, *Chironomus* (29) (48).

In addition to hemoglobin there are three other well-defined respiratory proteins. Chlorocruorin, which is most nearly related to hemoglobin, is found in the blood of certain worms,—the Sabellidae, Serpulidae, and Chlorhaemidae. Chlorocruorin is also a protein-hematin compound. The hematin, however, is not the same substance as that found in hemoglobin, being composed of a porphyrin distinct from that occurring universally in hemoglobin (32) (78) (79). Chlorocruorin has never been found in blood corpuscles, but occurs in solution in the blood and body fluids.

Hemerythrin is another respiratory protein containing iron which occurs in certain worms. This pigment does not appear to contain a hematin or a porphyrin and in certain other respects shares the characteristics of the hemocyanins (15) (53). Hemerythrin occurs in blood corpuscles, never in solution in the plasma. It is found in the blood of the Sipunculoidea, and in the polychaet, *Magelona*.

The hemocyanins, which are copper-protein compounds, show no chemical relation to hemoglobin and take no part in the respiratory function of the bloods which may be considered a part of the evolutionary series with which we are concerned.

The distribution of the hemoglobins in the animal kingdom is wide and, when considered from the point of view of evolutionary doctrine, puzzling. Hemoglobin is the universal respiratory pigment of the blood of vertebrates, being the only substance serving this function in the phylum and occurring in all its members with the exception of *Amphioxus*.[1] It is also the dominant respiratory pigment in the annelid worms, occurring in the majority of polychaets and echiuroids, in many oligochaets and in some leeches (16) (50) (51) (64). In addition to these groups, where its distribution is somewhat general, hemoglobin is found in a variety of isolated instances in other parts of the animal kingdom, as the summary in Table 1 indicates.

In addition to its occurrence in the blood, hemoglobin is found in other tissues, particularly the muscles and nervous tissues of many animals. In the former situation it occurs in some forms which do not contain hemoglobin in their blood, for example, in the pharyngeal muscles of certain gastropods whose blood contains hemocyanin as the respiratory pigment (50) (54).

The occurrence of hemoglobin in these random cases among natural groups whose other members as a whole do not possess this substance is surprising from the evolutionary point of view. One can only conclude that it has been possible for hemoglobin to arise more or less independently in a variety of different groups. The difficulty is somewhat relieved by the discovery that protophorphyrin, the principal specific building-stone of hemoglobin, is widely distributed throughout the animal kingdom and by the demonstration that cytochrome, which like hemoglobin appears to be a porphyrin derivative, and allied pigments are very general in their distribution among plants and animals (1) (28) (30) (46). These considerations make it extremely dangerous to relate the vertebrate blood to that of any invertebrate group, for it appears quite possible that hemoglobin may have arisen *de novo* in the blood of the ancestral vertebrates just as it appears to have in

Chironomus, *Planorbis*, and other isolated instances. On the other hand, if the distribution of hemoglobin in the animal kingdom has any relation to evolutionary history, then emphasis must be placed on the fact that the annelids are the only invertebrate group in which the occurrence of hemoglobin is really general. In attempting to compare conditions existing in the blood of the vertebrates with those of lower animals, we are forced to assume this point of view, since the worms are the only group affording pertinent data with which we may deal.

TABLE 1

Distribution of Hemoglobin in the Blood of Animals

PHYLUM	CLASS	GENUS
Platyhelminthes	Turbellaria	*Derostoma* (56) *Syndesmis* (58)
	Nemertinea	*Drepanophorus* (39) *Polia* (50)
Nemathelminthes	Nematoda	*Ascaris* (46)
Molluscoidea	Phoronida	*Phoronis* (50) *Phoronopsis* (60)
Echinodermata	Holothuroidea	*Thyone* (16) *Cucumaria* (16) (71) *Caudina* (48a) *Malpadia* (48a)
Annulata	Polychaeta	Many cases (16) (64)
	Oligochaeta	Many cases (16) (64)
	Echiuroidea	*Thalassema* (51) *Urechis* (61)
	Hirudinea	Some cases (16)
Arthropoda	Crustacea	*Daphnia* (50) *Cheirocephalus* (50)
	Insecta	*Chironomus* (50)
Mollusca	Pelecypoda	*Solen* (50) *Arca* (16) (66) *Cardita* (85) *Pectunculus* (16)
	Gastropoda	*Planorbis* (50)
Chordata	All vertebrates	(50)

The conditions existing in the blood of the polychaet worms have been reviewed amply by Romieu (64), who points out the great diversity of conditions occurring within this group. With a little freedom, his classification may be made to serve for the entire series in which hemoglobin occurs. Leaving out of account the large number of animals in which respiratory proteins do not occur, we find among the invertebrates two general situations which appear to include most cases. In those animals in which there exists a definite circulatory apparatus the respiratory pigment usually occurs in solution in the blood, which is sharply separated from a coelomic fluid, rich in leucocytes, and free from respiratory proteins. This is the most common situation among the polychaet worms and in the oligochaets. It is also the condition obtaining in those worms in which chlorocruorin occurs, except that in some at least of these forms chlorocruorin occurs also dissolved in the coelomic fluid, as in *Spirographis* and *Siphonostoma*.

In contrast there is a considerable number of animals in which the respiratory protein is enclosed in corpuscles and this situation occurs principally in those forms in which the vascular system is degenerate or lacking, the corpuscles being suspended in the coelomic fluid. This is the condition occurring in *Glycera*, *Capitella*, and *Polycirrus haematodes* among the polychaets, in the echiuroid *Urechis*, and in the sipunculoids *Phascolosoma* and *Sipunculus* in which the erythrocytes contain hemerythrin.

Two species of polychaets, *Terebella lapidaria*, and *Travisia Forbesii*, combine the two foregoing conditions. In these worms the vascular blood contains hemoglobin in solution, whereas the coelomic fluid is provided with abundant erythrocytes.

Finally, a single species of worm, *Magelona papillicornis*, exhibits a condition strictly comparable to that found in the vertebrates, that is, a blood confined to definite vessels in which the respiratory pigment is carried in blood corpuscles. The resemblance to the vertebrate condition is furthered by the fact that in *Magelona* alone, among all invertebrates, the blood corpuscles appear to be anucleated. The comparison to the vertebrate condition breaks down entirely, however, when it is considered that in *Magelona* the respiratory protein is hemerythrin.

The condition observed in *Phoronis*, *Phoronopsis*, and certain Nemertinea may best be classed with *Magelona*, for in these forms the blood is confined to definite vessels and contains erythrocytes bearing hemoglobin. The vascular system of the Phoronidae, however, is organized on somewhat different lines from those followed in the case of the worms and vertebrates.

From the foregoing it appears that although the situation under which hemoglobin occurs in the body fluids of the invertebrates is most varied, conditions which are strictly comparable to those found in the vertebrate series are rare and occur in rather isolated cases.

The general character of the erythrocytes found among the vertebrates is well known. It will suffice to recall that in all mammals, except in the camels, they are circular disc-shaped bodies. In the camels they are flattened ellipsoids. In the mammals they contain no nuclear material; in all other vertebrates they possess well-marked nuclei. In the birds, reptiles, amphibians and in all fishes except the cyclostomes, the erythrocytes are flattened ellipsoids; in the cyclostomes the shape is discoid (57).

The erythrocytes of the invertebrates are nucleated bodies, spherical in *Thalassema* (51) and *Urechis* (61), or flattened discs of nearly circular outline, in *Terebella lapidaria*, *Glycera* (64), *Phascolosoma*, *Arca*, and *Thyone* (16a). The corpuscle is surrounded by a definite membrane which appears to enclose a distinctly fluid cytoplasm. In addition to the dissolved hemoglobin, the cytoplasm frequently contains highly refractive bodies, which Romieu considers to be fatty in nature, and also colored granules. These are thought to be accumulated waste materials by Romieu, who identifies them as a uratic pigment. Similar bodies are frequently very numerous in the erythrocyte of *Urechis*. Baumberger and Michaelis (8) consider the pigment to be hematin in this form and present evidence that the accumulation of hematin is utilized in forming the pigments of the egg. Romieu also presents a similar view with regard to the utilization of the pigments of the erythrocytes of *Terebella*. Rather extensive patterns of reticulation, resembling those of vertebrate erythrocytes, appear in the corpuscles of *Glycera*, *Phascolosoma*, *Arca*, and *Thyone* on treatment with brilliant cresyl blue (16a).

The invertebrate erythrocyte appears in general to be less highly differentiated than the vertebrate red blood corpuscle. Romieu's investigations indicate that the red cell found in the coelomic fluid of worms is strictly homologous to the vertebrate erythrocyte, the young corpuscle passing through all the phases which are known among the vertebrates. Jordan (40) believes that if one assumes a continuous evolution from invertebrates to vertebrates, a transition can be traced from segmented worms to cyclostome fishes in which the lymphogenous organ of invertebrates may be regarded as a very primitive spleen.

The apparent relation between the erythrocyte of the vertebrate blood and the corpuscle of the coelomic fluid in the invertebrates deserves some emphasis. Physiologists have tended to consider the occurrence of hemoglobin in solution in the blood of worms as representing a primitive condition and as such the evolutionary forerunner of blood containing erythrocytes. This view is definitely taken by Romieu (64), who suggests that the development of the coelomic erythrocyte has rendered the vascular system useless and has led to its degeneration in many forms. It has been maintained that the raison d'être of the erythrocyte is to permit hemoglobin to exist in the blood in quantities greater than would be possible were it dissolved directly in the circulating fluid (3). While it cannot be denied that the blood of the higher vertebrates contains more hemoglobin than could be dissolved in a fluid adapted to circulate in the vascular system, this possibility does not appear to have been realized in the invertebrate stage of evolution, for *Arenicola* and *Spirographis*, which carry their respiratory pigments in solution, have blood with a greater oxygen capacity than *Urechis* and the other invertebrate forms in which oxygen is transported in corpuscles (61). When these considerations are taken in connection with the fact that red blood corpuscles exist in such primitive forms as the nemerteans and *Phoronis*, one may properly question whether the occurrence of hemoglobin in solution in the blood of worms does not represent a rather specialized development. It seems significant in this connection that when hemoglobin occurs in solution it is always confined to a closed vascular system. It is now understood that the proteins of the plasma of the blood of the higher vertebrates play an important rôle in the hydrostatic equilibrium which determines the exchange of water through the capillary wall. It is known that fluids free from protein or other substances of large molecular size cannot be retained within the vascular system. Is it not probable that the hemoglobin dissolved in the blood of the worms serves a function in counterbalancing the effect of the pressure developed within the blood vessels analogous to that exerted by the plasma proteins of the vertebrates? This action would not occur if the hemoglobin were retained in corpuscles unless other specialized serum proteins were developed to serve the purpose.

These considerations suggest that the hemoglobin which exists in solution in the blood of the invertebrates supplies a factor necessary in the hydrostatic equilibrium on which the existence of vascular blood depends. In the vertebrates the general occurrence of plasma proteins fulfills this requirement and makes it possible for hemoglobin to be confined to corpuscles suspended in the vascular fluid.

Certain quantitative facts appear to support this view. First, there is evidence of an increase in the concentration of plasma proteins in the evolutionary series. The body fluids of invertebrates are in general extremely poor in protein except for those cases where hemocyanin and the other respiratory proteins occur in solution. Among the worms, for example, there are no proteins in the coelomic fluid of *Echiurus pallasii* (68). In *Sipunculus* the concentration of protein in the coelomic fluid is 0.11 per cent (17); in *Urechis* 0.11 per cent (45). Among the vertebrates a progressive increase in the concentration of plasma protein occurs, the following values being recorded; skate, 2.4–3.1 per cent (20); dogfish, 2.2–4.4 per cent; goosefish, 1.4–2.2 per cent; menhaden, 0.72–2.9 per cent; bullhead, 3.9–4.8 per cent (52a); lungfish, 5.5 per cent (69); frog, 1.5–4.29 per cent (13); crocodile, 3.69 per cent (19); alligator, 4.4–5.8 per cent (2); snapping turtle, 4.8 per cent (35); dog, 6.1–6.7 per cent (21); man, 6.5 per cent (35). The increasing values appear to parallel the development of a cardiovascular system capable of containing blood at increasing pressures. In the second place, when hemoglobin occurs in solution in the blood, its concentration is comparable to that of the serum proteins in the lower vertebrates. It is estimated that the blood of *Planorbis* contains 1.5 per cent hemoglobin, that of *Arenicola* 3.25, that of *Lumbricus* 3.7 per cent (41). It is also pertinent that in the blood of *Syllidiens*, which contains no hemoglobin, the vascular blood contains a much larger quantity of protein than the perivisceral lymph (64). A more extensive investigation of the plasma proteins in the invertebrates and lower vertebrates is very desirable from this point of view.

THE QUANTITY OF HEMOGLOBIN IN THE BLOOD AND IN THE ERYTHROCYTE

Practical indication of the quantity of hemoglobin contained in the blood of various animals is given by measurements of the amount of oxygen with which it will combine when in equilibrium with atmospheric air. Representative data for various animals are presented in Table 2. In general there is an increase in the oxygen capacity of the blood in the course of the evolutionary series, the highest values of about twenty volumes per cent being found in the birds and mammals, the lowest values among the invertebrates. Within each group there is considerable variation which may be related directly to the activity of the various species. This is particularly well shown among the fishes (33). Table 2 also shows the way in which the volume of the blood occupied by cells tends to increase in the course of the evolutionary series. The ratio of

TABLE 2

The Oxygen Capacity and Cell Volume of the Blood of Various Animals

SPECIES	OXYGEN CAPACITY OF BLOOD	CELL VOLUME	OXYGEN CAPACITY OF 100 CC. CELLS
	vol. per cent	*per cent*	*vol. per cent*
Mollusks:			
Cardita sulcata (85)	1–2		
Pectunculus violaceus (85)	1–2		
Arca inflata (66)		6.5	
Planorbis corneus (10) (52)	1.0–2.5	None	
Insects:			
Chironomus (52)	6	None	
Worms:			
Urechis caupo (61)	2.7–7.2	18–40	9.3–17
Glycera siphonostoma (85)	2.6–3.0		
Arenicola marina (10)	8.4–9.7	None	
Spirographis Spallanzanii (32)	8.1–10*	None	
Fishes:			
Skate, *Raia ocellata* (20)	4.2–6.0	20	30
Dogfish, *Mustelus canis* (25)	5.5–7.8		
Goosefish, *Lophius piscatorius* (65)	5.1	15.5	33
Toadfish, *Opsanus tau* (65)	6.2	19.5	32
Puffer, *Spheroides maculatus* (65)	6.8	17.5	39
Scup, *Stenotomus chrysops* (65)	7.3	32.6	23
Sea robin, *Prionotus carolinus* (65)	7.7	24	32
Carp, *Cyprinus carpio* (23) (80)	11.5–16.8	40	35
Mackerel, *Scomber scombrus* (65)	15.8	37	43
Eel, *Anguilla japonica* (43)	10.2–15.6	31–41	35
Amphibians:			
Amphiuma tridactyla (67)	2.5–8.4	14–28	25
Rana esculenta (23) (84)	13.5–23	41	33
Reptiles:			
Snapping turtle, *Chelydra serpentina* (35)	5.9	20.4	29
Tortoise, *Pseudemys concinna* (70)	6.6–11	9.8–22	50
Alligator (38)	12	14.5	83
Crocodile, *Crocodilus acutus* (19)	8–10	18–24	43
Birds:			
Sparrow (23)	14.5	37	39
Serin (23)	14	36	39
Pigeon (23)	21	53	40
Surf scooter (60)	22	45	47
Crow (23) (83)	17–22	54	40
Owl, *Syrnium aluco* (83)	19		
Mammals:			
Man (24)	21	46	46
Horse (24)	16.7	36	47
Sheep (24)	15.8	39	41
Rat (24)	18.7	47	40
Sea lion, *Eumetopias stelleri* (31)	19.8	29	68
Porpoise (70a)	42–45		
Porpoise, *Phocaena phocaena* (32b)	22.2	35	63

* Blood contains chlorocruorin.

oxygen capacity to the volume of cells in a unit quantity of blood, tabulated in the fourth column, shows how the concentration of hemoglobin varies in the red corpuscles of the different groups. These data exhibit a very definite trend. The concentration of hemoglobin in the erythrocyte of *Urechis* is much less than that observed in any vertebrate. The values are relatively constant among the fish and amphibians and are highest in certain reptiles, birds, and mammals. The highest values are found in the aquatic mammals. (The exceptionally high value obtained for the alligator would appear to require confirmation in view of its dissimilarity from that characterizing the crocodile and other cold-blooded vertebrates.) The progressive morphological differentiation which the erythrocytes show throughout the animal series is accompanied by a definite increase in the concentration of hemoglobin within them.

THE EQUILIBRIUM BETWEEN HEMOGLOBIN AND OXYGEN

The equilibrium between oxygen and the hemoglobin of the blood is commonly expressed by the so-called "oxygen dissociation curve" in which the amount of oxygen combined with the blood, expressed as a fraction of the total oxygen capacity, is plotted against the partial pressure of oxygen with which the blood is in equilibrium (Figs. 1 and 2). Although more than half a dozen theories have been proposed, the interpretation of the exact shape of such curves is not altogether clear (5) (27). For purposes of description the oxygen dissociation

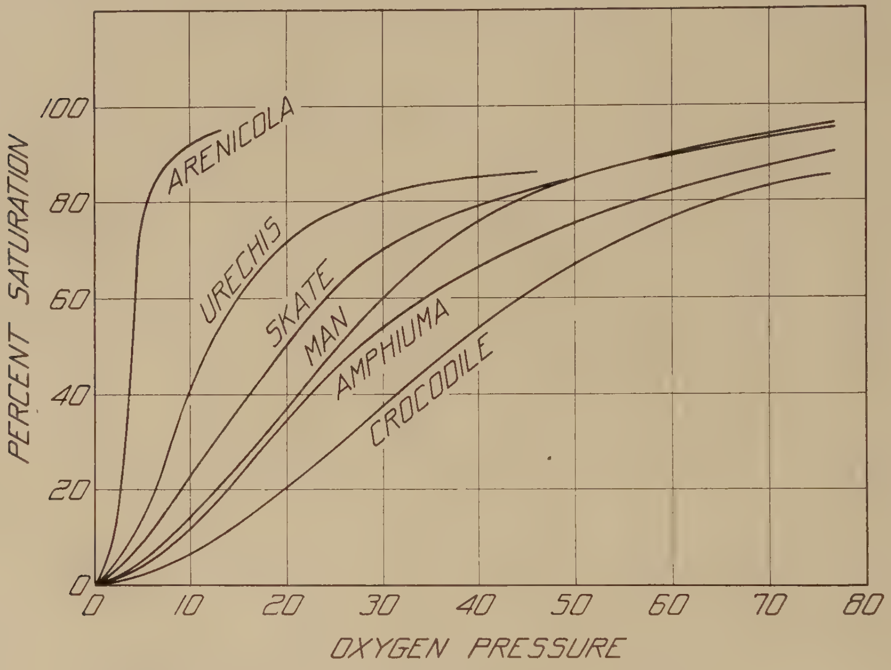

Fig. 1. Oxygen Dissociation Curves of Typical Sigmoid Shape

Ordinate, percentage of saturation with oxygen; abscissa, oxygen pressure in mm. Hg. *Arenicola* (6), 20°C., pH 6.9; *Urechis* (61), 19°C., pH 7.1; skate (20), 10.4°C., pH 7.8; man (35), 38°C., pH 7.47; *Amphiuma* (67), 26°C., carbon dioxide pressure 43 mm.; crocodile (19), 29°C., pH 7.2.

curves of most bloods are defined with sufficient precision by Hill's (37) equation

$$y = 100 \frac{Kx^n}{1 + Kx^n}$$

in which y is the percentage of the hemoglobin oxygenated at the partial pressure of oxygen, x. The general shape of the oxygen dissociation curve is related to the value of n. For a hyperbolic curve, n is 1.0; at higher values of n (above about 1.4) the curve acquires a double inflexion or S-shape which becomes more marked as the value of n increases.

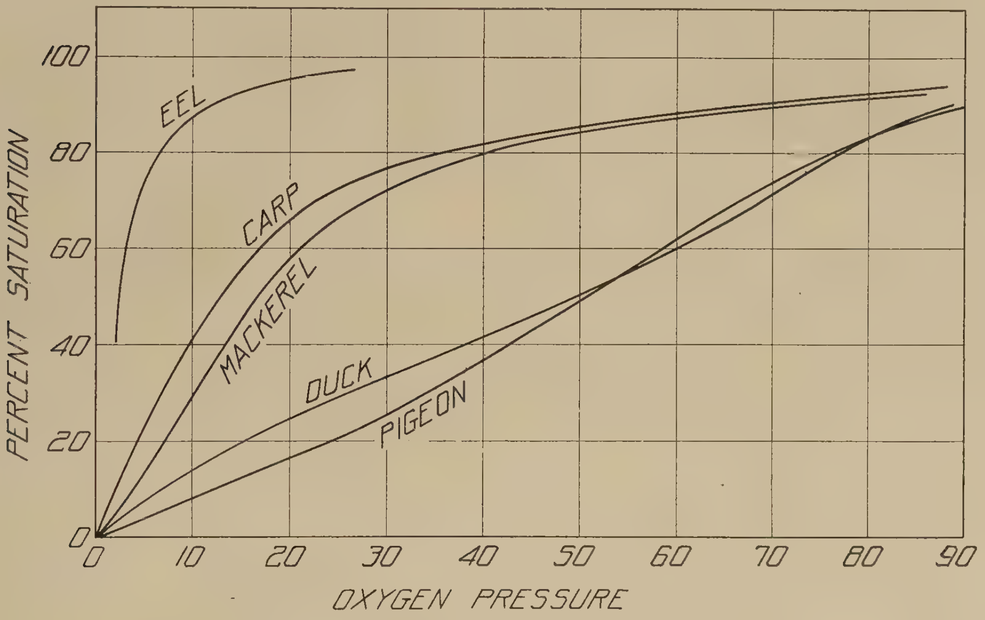

Fig. 2. Oxygen Dissociation Curves of Atypical Shape

Ordinate, percentage of saturation with oxygen; abscissa, oxygen pressure in mm. Hg. Eel (44), 17°C., carbon dioxide pressure not given; carp (80), 18°C., carbon dioxide pressure 30 mm.; mackerel (65), 20°C., carbon dioxide pressure 1 mm. Hg.; duck (81), 42°C., carbon dioxide pressure 40 mm.; pigeon (81), 42°C., carbon dioxide pressure 40 mm.

The shape of the oxygen dissociation curve, as indicated by n, appears to be affected little or not at all by temperature (11) (81) and by changes in hydrogen ion concentration or carbon dioxide pressure (5) (27). This fact simplifies the comparison of the shape of the oxygen dissociation curves of different species, since the differences in the conditions under which the measurements have been carried out may be neglected. The value of n characteristic of different bloods varies considerably as the following table shows:

	n
Skate (20)	2.0
Eel (44)	1.0
Carp (80)	1.3
Amphiuma (67)	1.8
Turtle (70)	1.5
Man (5)	2.2
Pig (11)	2.0–2.6
Ox (11)	2.3–3.0

The bloods of the great majority of animals show a distinctly S-shaped oxygen dissociation curve, as indicated by the higher values of n. This is true of the mammals, the crocodile (19), *Amphiuma*, the skate, and the worms *Urechis* (61) and *Arenicola* (6). The oxygen dissociation curves of the blood of fish appear to be somewhat exceptional in that the sigmoid character of the curve is less pronounced (in the mackerel) or is lacking as in the sea robin, toadfish (65), and carp (80). The blood of the eel is said to have a purely hyperbolic oxygen dissociation curve, but the evidence for this cannot be considered convincing. Figure 2 illustrates the form of some of these atypical oxygen dissociation curves.

Studies of the oxygen dissociation curves of fishes made since the foregoing was written display a number of unique conditions not previously described and throw some light on the nature of the atypical oxygen dissociation curves (32c).

The oxygen dissociation curve of the birds is also exceptional, and cannot be described by Hill's equation (81) (82). That of the duck has the curious shape shown in Fig. 2. Those of the pigeon and goose are intermediate in form between that of the duck and the more typical sigmoid curve observed in most vertebrates. Such curves have not been described for other bloods containing hemoglobin. They are, however, characteristic of the bloods of the horse-shoe crab, *Limulus*, and the gastropod, *Busycon*, which contain hemocyanin (60).

The comparative study of the equilibrium of blood with oxygen is complicated by the variety of factors which influence the degree of oxygenation which will be attained under any given oxygen pressure. This relation is defined by the value of the oxygen dissociation constant K in Hill's equation. Certain of these factors are inherent in the chemical properties of the medium in which hemoglobin exists in the blood. Others, such as temperature, are determined purely by the environmental conditions in most forms, while some factors, such as carbon dioxide tension and hydrogen ion concentration, are influenced alike by physiological and environmental considerations. It is frequently difficult to compare the data obtained by various observers because each has been concerned with a somewhat different aspect of the problem and it is infrequent that data are obtained under strictly comparable conditions. It is consequently desirable to consider some of the factors affecting the equilibrium of oxygen with hemoglobin with a view to discovering to what extent these difficulties may be overcome.

The effect of temperature upon chemical equilibria is expressed by an equation derived from the van't Hoff isochor:

$$\frac{K_2}{K_1} = e^{-q\,(T_2 - T_1)/RT_1T_2}$$

in which K_1 and K_2 are the equilibrium constants for the reaction at absolute temperatures T_1 and T_2 and q expresses the heat evolved when one gram molecule of the reaction product is formed. This equation appears to supply a satisfactory basis with which to deal with variations in the equilibrium of hemoglobin and oxygen under the influence of temperature.

The value of q includes not only the heat evolved when hemoglobin combines with oxygen, but, as commonly measured, the heat of solution of oxygen and such other heat exchanges as accompany the altered equilibria which occur when blood is oxygenated. The latter are due chiefly to the fact that hemoglobin becomes a stronger acid when oxygenated and in consequence a new equilibrium is established in the distribution of base between hemoglobin, bicarbonate and perhaps other weak acids. Since it has been shown that in the case of the blood of the pig, ox (11), pigeon, duck, and goose (81) (82) the shape of the oxygen dissociation curve is unchanged by variations in temperature, in practice the reciprocal of p_{50}, the oxygen tension at which the blood is half saturated with oxygen, may be substituted for the equilibrium constant. The data may be expressed by plotting the logarithm of p_{50} against the reciprocal of the absolute temperature when it is found that the experimental points fall on a line which is straight, or nearly so, and with a slope which is determined by q. When blood is studied under suitable conditions, q has very nearly the same value in a considerable number of species. This is brought out by the fact that the various curves illustrated in Fig. 3 have about the same slope. Such similarity is only to be expected if the heat of reaction between hemoglobin and oxygen is about the same in all species and provided the conditions are such that the accessory reactions accompanying oxygenation absorb an equal fraction of the heat of reaction in each case. These facts point to a general similarity in the blood of different forms in respect to the thermodynamic changes involved in the combination of hemoglobin with oxygen. When the experiments are carried out at diminishing carbon dioxide pressures (7) (81), in the absence of carbon dioxide (11), or at constant hydrogen ion concentration (26), temperature changes have a larger effect upon the value of the equilibrium constant, and the heat of reaction is larger when measured directly, than is the case in experiments done under constant carbon dioxide pressures of normal physiological value. This is explained by the diminished degree to which heat is absorbed

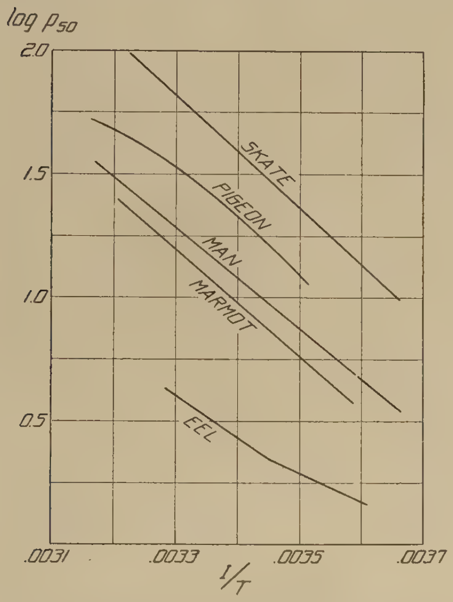

Fig. 3. The Effect of Temperature on the Tension of Oxygen at Which the Blood is Half Saturated with Oxygen

Ordinate, log p_{50}, the logarithm of the tension of half saturation. Abscissa, $1/T$, the reciprocal of the absolute temperature. Skate (20), CO_2 tension 1 ± 0.5 mm.; pigeon (81), CO_2 tension 40 mm.; man (11), CO_2 tension 40 mm.; marmot (26), CO_2 tension 40 mm.; eel (44), CO_2 tension not given.

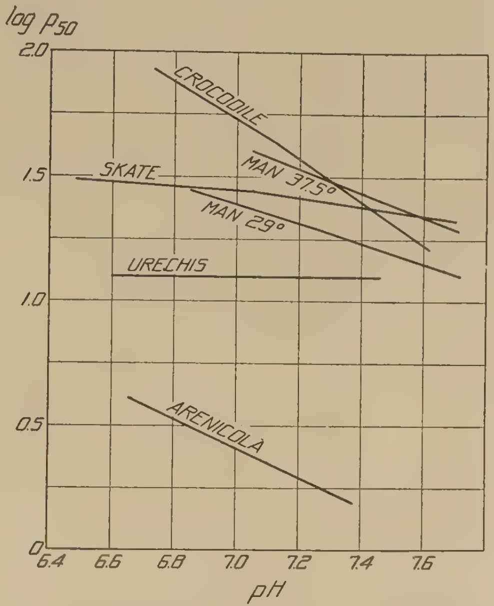

Fig. 4. The Effect of Hydrogen Ion Concentration upon the Pressure of Oxygen at Which Blood is Half Saturated with Oxygen

Ordinate, log p_{50}, the logarithm of the tension of half saturation; abscissa, hydrogen ion concentration expressed in pH units. Crocodile (19), 29°C.; man (35), 37.5°C. and (19) 29°C.; skate (20), 10.4°C.; *Urechis* (61), 19°C.; *Arenicola* (6), 20°C.

through changes in the quantity of base combined with the hemoglobin.

The effect of hydrogen ion concentration upon the equilibrium of various bloods with oxygen is more difficult to deal with. The hydrogen ion concentration, depending as it does on the ratio of acids and bases in the blood and upon the equilibria which determine the distribution of electrolytes between corpuscles and plasma, is defined by a multiplicity of factors. One may arrive at an approximation to the hydrogen ion concentration if one knows the pressure of carbon dioxide in the system and the amount of carbon dioxide bound as bicarbonate. Unfortunately, not many studies of the blood of the lower animals provide these data simultaneously, attention usually being limited to the effect of carbon dioxide pressures upon the oxygen dissociation curve without reference to the bicarbonate content. However, a limited amount of data is available which enables one to compare the effect of hydrogen ion concentration upon the equilibrium of different kinds of blood with oxygen. These data are summarized in Fig. 4, in which the logarithm of the oxygen tension at which the hemoglobin is half saturated with oxygen is plotted against the pH of the blood. The facts summarized in this figure make it clear that the various bloods vary distinctly in regard to the effect of hydrogen ion concentration upon the equilibrium of blood with oxygen. In some bloods, as that of the skate at acid reactions, and that of *Urechis*, the hydrogen ion concentration has little or no effect on the oxygen equilibrium. In most of the bloods which have been studied the effect is marked, its magnitude differing from species to species. If the data presented in Fig. 4 are corrected to some uniform temperature employing the principle set forth above, the agreement among the various species is not improved. It seems probable that these variations are due to specific differences in the hemoglobin of the different animals. From the available data it is impossible to see any definite tendencies which may be related to the systematic classification of the animals.

A somewhat special relationship between hydrogen ion concentration and the combination of hemoglobin with oxygen appears in the blood of certain teleost fishes. From studies upon the blood of the mackerel, sea robin, and toadfish, Root (65) has concluded that increasing concentrations of carbon dioxide not only influence the oxygen dissociation constants of the blood but bring about a decided decrease in the oxygen capacity of the hemoglobin. It appears as though certain of the prosthetic groups concerned in binding oxygen to the hemoglobin molecule were inactivated. The effect is also produced by lactic acid so that it cannot be attributed specifically to carbon dioxide. Similar phenomena do not appear to have been recorded for the bloods of other groups.

It is apparent that the bloods of various animals differ markedly in the conditions governing the equilibrium with oxygen. This fact may be most simply explained on the assumption that the hemoglobins of the different species are chemically distinct. For this view there is some evidence of a spectroscopic, crystallographic and chemical sort (5) (34) (48a) while immunological experiments indicate that the carbon monoxide hemoglobin from different mammals is species specific (9).

The nature of the chemical differences in different hemoglobins is, however, still quite illusory. Since the porphyrin portion of all hemoglobins appears to be the same (29) (48), it has generally been suspected that the specific qualities of hemoglobin depend upon the globin fraction of the molecule. Investigations of globin as yet do not appear to have demonstrated the nature of such specific differences if they exist (63).

It has also been suggested that the specific differences may be due to the occurrence of hemoglobin within semi-permeable corpuscles which enables a characteristic chemical environment to be secured for the hemoglobin of each species and thus determines its specific properties in each organism. Against this view it may be pointed out that the properties of the blood of the dog are very similar to those of the blood of man in spite of the great differences in the composition of the corpuscular electrolytes in these species (21). The two views are not mutually exclusive. In the absence of any studies of the properties of the purified proteins of the lower forms it is impossible to evaluate the importance of either possibility.

THE EQUILIBRIUM OF THE BLOOD WITH CARBON DIOXIDE

Carbon dioxide is transported in the blood as bicarbonate which results from the union of this gas—as carbonic acid—with base derived from hemoglobin, serum proteins and other buffers. It is not surprising consequently that the carbon dioxide dissociation curves of most animals are similar in form, and differ chiefly in the quantity of bicarbonate formed under a given carbon dioxide pressure. The curves shown in Fig. 5 illustrate this. It should be noted that the height of the curves from the base line follows in general the order of increasing hemoglobin content—as indicated by the recorded oxygen capacities of the blood.

The blood of a certain group of animals exhibits carbon dioxide dissociation curves which differ distinctly from the typical form. These are the bloods of the turtle (70), frog (84), *Amphiuma* (67), and carp (80). The carbon dioxide dissociation curves of these forms illustrated in Fig. 6. are all very much higher and flatter than those of the bloods exhibiting the more usual condition and indicate a greater ability to combine with carbon dioxide than would be expected from the hemoglobin content. It has been shown that this form of curve is due to the retention in the blood of an unusually large quantity of bicarbonate (67) (70) relative to the hemoglobin concentration. The effect is to be attributed to some peculiarity in the mechanism whereby the electrolyte content of the blood is regulated.

The buffering of the blood may be more precisely analyzed with the aid of the relation

$$\beta = \frac{-\Delta\ \mathrm{BHCO_3}}{\Delta\ \mathrm{pH}} = \frac{-\Delta\ \mathrm{BHCO_3}}{\Delta\ \log \frac{\mathrm{BHCO_3}}{\mathrm{H_2CO_3}}}$$

where the buffer value, β, is given by the ratio of the change in the bicarbonate content to the accompanying change in pH when the carbon dioxide pressure is increased (72). In the invertebrates it appears that the respiratory protein itself is the principal, if not the sole buffer substance. In *Limulus* the hemocyanin accounts for practically the total buffer effect (62). In *Urechis* the coelomic fluid appears to contain practically no buffers. The total buffer action is exerted by virtue of the corpuscles and their buffer value is equal to that of a quantity of human hemoglobin having an equivalent oxygen content (61). In the vertebrates also the buffer value of the blood as a whole when compared on the basis of equivalent oxygen capacity is approximately the same in the case of the skate (20), turtle (70), crocodile (19), dog (20), and man

(19). This general relation is reasonable, from the physiological standpoint, for under ordinary circumstances the carbon dioxide production of animals is nearly equal to oxygen consumption so that the needs for transport of the two gases go hand in hand. Table 3 summarizes the buffer value of the hemoglobin in the corpuscles of a number of different animals.

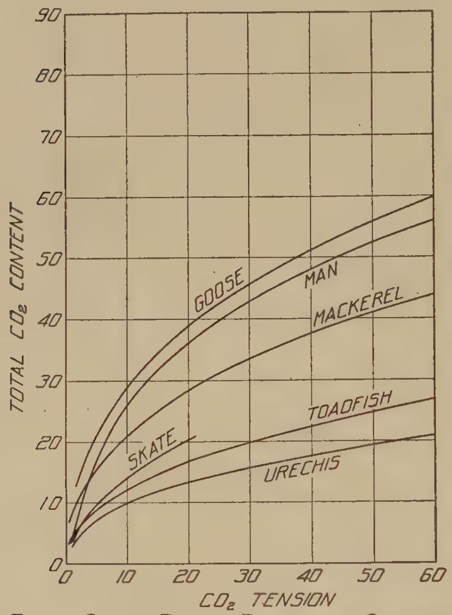

FIG. 5. CARBON DIOXIDE DISSOCIATION CURVES OF TYPICAL SHAPE

Ordinate, total carbon dioxide content measured in volumes per cent; abscissa, partial pressure of carbon dioxide measured in mm. Hg.

	Temperature °C	*Oxygen capacity vol. per cent*
Goose (83)	40	20
Man (35)	38	20
Mackerel (65)	20	15.8
Skate (20)	25	4.2–6.0
Toadfish (65)	20	6.2
Urechis (61)	18.5	3.9

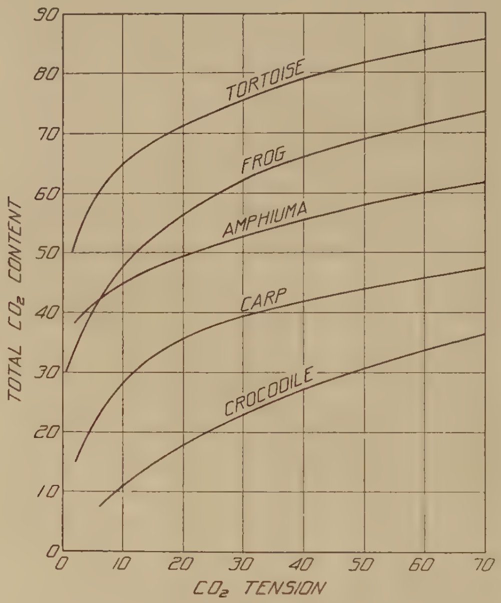

FIG. 6. CARBON DIOXIDE DISSOCIATION CURVES OF ATYPICAL SHAPE

Ordinate, total carbon dioxide content measured in volumes per cent; abscissa, partial pressure of carbon dioxide measured in mm. Hg.

	Temperature °C	*Oxygen capacity vol. per cent*
Tortoise (70)	25	ca. 9
Frog (84)	15	ca. 10
Amphiuma (67)	24	ca. 5
Carp (80)	15	ca. 9
Crocodile (19)	29	9.25

The blood of vertebrates contains serum proteins which contribute definitely to the buffer value of the blood. It has been pointed out that the serum proteins are present in increasing concentration in the blood of the higher members of the vertebrate series. The relative part played by the serum proteins in buffering the blood against carbon dioxide is indicated in Table 4. The last column in this table indicates the relation of the buffer value of the serum proteins to their concentration in the blood and shows that the serum proteins in the skate and crocodile are more effective buffers than those in man. In this way they compensate in part for their lower concentration. Serum proteins are inferior buffers compared to hemoglobin as the recorded buffer values show.

In the Mammalia a large portion of the transport of carbon dioxide from the tissues to the lungs is effected as the result

TABLE 3

Buffer Values of Hemoglobin in Corpuscles at Physiological Reactions

SPECIES	BUFFER VALUE
	milliequivalents per gram hemoglobin
Urechis caupo, 19° (61)	0.169
Crocodilus acutus, 29° (19)	
Oxygenated	0.208
Reduced	0.143
Man, 29° (19)	
Oxygenated	0.183
Reduced	0.159
Man, 37.5° (18)	
Oxygenated	0.186
Reduced	0.166
Horse, 38° (74)	
Oxygenated	0.158
Reduced	0.147

In computing these values it is assumed that the equivalent weight of hemoglobin is 16,700 in all three species. The buffer value of the hemoglobin of the horse is based on studies of crystalline hemoglobin. That of other species is deduced from the study of whole blood.

TABLE 4

Buffer Values of Whole Blood, Separated Serum and Serum Proteins

SPECIES	TOTAL BLOOD OXYGENATED	TOTAL SERUM SEPARATED	SERUM PROTEINS
	milliequivalents per liter	*milliequivalents per liter*	*milliequivalents per gram protein*
Urechis caupo, 19° (61)	4.9	0	0
Skate, *Raia ocellata*, 25° (20)		4.63–6.48	0.193–0.209
Crocodile, *Crocodilus acutus*, 29° (19)	18.2	4.87	0.132
Dog, 37.5° (21)			0.093
Horse, 38° (75) (76) (77)	25.26		0.068–0.104
Man, 37.5° (36)	26.2	7.5	0.109

The buffer values for serum proteins of the horse are based on dialyzed serum (0.068) and purified serum proteins (0.104). The values for other animals are deduced from studies of plasma without correction for small amounts of other buffer substances.

of the fact that oxyhemoglobin is a stronger acid than reduced hemoglobin. At any carbon dioxide tension, reduced blood will combine somewhat more of this gas than will oxygenated blood. A similar effect has been observed in a large number of vertebrate bloods, although in some species it is necessary to concentrate the corpuscles before the effect is large enough to be clearly measurable. The effect is absent, or at least undetectable, in the blood of *Urechis* and of the skate. In these forms the reciprocal phenomenon, in which the presence of carbon dioxide affects the equilibrium between hemoglobin and oxygen, is absent (in *Urechis*) or very slightly developed (in the skate). Table 5 shows the magnitude of the change in bicarbonate content with change in oxygen content of the blood of a variety of animals. It is clear that this property of the blood varies definitely in different animals. Since the relation is a function of the properties of hemoglobin, it is probable that the differences observed in the various species are due to specific differences in their hemoglobins.

Although hemoglobin is the principal buffer substance concerned with the transport of carbon dioxide in the blood, this gas, unlike oxygen, is not confined to the corpuscles, but is distributed according to well-recognized principles between the corpuscles and plasma (73). The hemoglobin within the corpuscle is able to contribute to the buffer value of the plasma by reacting with anions, chiefly chloride, which migrate into the corpuscles from the plasma. The excess of cations thus set free in the plasma can consequently combine with carbonic acid to form bicarbonates. A similar phenomenon appears to occur in the blood of *Urechis* (61), the turtle (70), the crocodile (19), and the hen (14) and is doubtless a general phenomenon. In these forms the facts have not been studied in detail, and the quantitative aspects of the factors governing the distribution of anions and cations between corpuscles and plasma are not altogether clear. Since these animals all possess a relatively small volume of corpuscles, the phenomenon is of particular importance in that it allows most of the carbon dioxide to remain in the plasma where it is distributed through a volume large in comparison to that of the corpuscles which retain the hemoglobin.

TABLE 5

Effect of Oxygenation upon the Carbon Dioxide Content of Bloods at Constant Hydrogen Ion Concentration

The values are for a pH of 7.2 to 7.4 or when log $\frac{BHCO_3}{H_2CO_3} = 1.0$.

SPECIES	$-\frac{\Delta BHCO_3}{\Delta O_2}$
Urechis caupo (61)	0
Skate, *Raia ocellata* (20)	0
Toadfish, *Opsanus tau* (65)	0.45
Sea robin, *Prionotus carolinus* (65)	0.45
Mackerel, *Scomber scombrus* (65)	0.70
Amphiuma tridactyla (67)	0.54
Tortoise, *Pseudemys concinna* (70)	0.32
Snapping turtle, *Chelydra serpentina* (35)	0.22
Crocodile, *Crocodilus acutus* (19)	1.05
Caiman fuscus (19)	0.56
Goose (82)	0.50
Horse (75)	0.55
Man (35)	0.49

RÉSUMÉ

The properties of the blood of the vertebrates and their forerunners display great variety in their quantitative relationships. In reviewing them, certain general tendencies have appeared. The variety of situations in which hemoglobin occurs in the invertebrates disappears in the vertebrates, among which the hemoglobin is universally confined within corpuscles in the blood. The morphological differentiation of the erythrocyte, which continues through the vertebrate series, is accompanied by an increased concentration of hemoglobin within the corpuscle. This tendency, coupled with an increase in the quantity of corpuscles in the blood, has led to a greater oxygen-carrying capacity in the more highly developed members of the series, and in the more active representatives of the lower groups. In turn the carbon dioxide-combining capacity and buffering powers of the bloods have increased with increasing hemoglobin content.

Throughout the series there has been a tendency for the plasma proteins to increase. This development is probably related to the hydrostatic conditions within the blood vessels and parallels an increase in the blood pressure in the various groups, but it contributes somewhat to the buffer power of the blood. A special tendency of the blood to contain a large quantity of bicarbonate appears in certain of the lower freshwater vertebrates, giving them a specialized type of carbon dioxide dissociation curve.

Throughout the series the respiratory properties of the blood are dependent upon hemoglobin, and the general properties of this substance are displayed in general by all forms. Thus the effect of temperature on the oxygen equilibrium and the buffer action of hemoglobin appear to be very similar throughout. All hemoglobins, with the exception of that of *Urechis* and under some circumstances the skate, exhibit the remarkable reciprocal relation between oxygen, carbon dioxide, and the products of their reaction with hemoglobin.

Quantitatively, however, there is clear individuality in the behavior of the hemoglobin of the different species. This appears in the shape of the oxygen dissociation curve, particularly in birds and fishes; in the oxygen pressures necessary to cause the hemoglobin to unite with oxygen; in the magnitude of the effect of altered hydrogen ion concentration on these pressures; and reciprocally in the effect of oxygenation and reduction on the quantity of carbon dioxide with which the blood will combine.

To what extent may these developments, quantitative or qualitative, be related to the special physiological problems faced by the respiratory mechanism of the various animals?

PHYSIOLOGICAL CONSIDERATIONS

Early students of the distribution of hemoglobin among the invertebrates were struck with its sporadic occurrence and pointed out that the possessors of this substance were animals which lived in places in which the oxygen supply is limited. It was suggested that hemoglobin developed in response to this situation, and that the substance provided a useful reservoir of oxygen for use in times of need. This suggestion was examined by Miss Leitch (52), who showed that in *Planorbis* the supply of oxygen combined with hemoglobin was only sufficient to last the animal for three minutes and consequently could not be considered seriously as a provision for prolonged anaërobic existence. The oxygen storage theory has been reviewed by Barcroft (6), who pointed out that the oxygen combined with *Arenicola* blood is sufficient to meet the requirements of the worm for an hour or thereabouts and will thus serve to tide over the period when the animal closes its burrow at low water. A somewhat similar situation occurs in *Sipunculus* (12) and *Urechis* (61). In the latter the oxygen content of the blood is sufficient to last about an hour, and probably serves during the prolonged periods of rest when respiratory activity is interrupted.

Borden (10) has re-examined the matter in a careful study of *Planorbis corneus* and *Arenicola marina*. She finds that the blood of *Planorbis* can combine enough oxygen to last 18 minutes and that this together with the air entrapped in the lung is sufficient to enable the snail to endure an hour's subjection to oxygen deficiency. She concluded that in both *Planorbis* and *Arenicola* the function of the hemoglobin is to carry oxygen when the animals are subjected to low oxygen pressures, but that the importance of hemoglobin as a temporary store is not negligible in either species. There can be no doubt that the oxygen capacity of the blood is important in enabling animals to resist short periods of suspended respiration, as in whales and diving birds, but it is significant that high oxygen capacities are developed in those forms which are most dependent on an abundant oxygen supply, as in the pelagic fish, birds, and mammals, and not in those which dwell in poorly aërated regions. Animals which are resistant to oxygen-lack frequently are devoid of respiratory pigment and their resistance appears to depend rather on other qualities of their tissues.

The proper significance of hemoglobin in relation to the oxygen of the environment was clearly shown by the experiments of Miss Leitch on *Planorbis* and

Chironomus. She found that in these forms the hemoglobin is fully oxygenated when the animals are in well-aërated water. Under such circumstances the solubility of oxygen in the body fluids is sufficient to meet all metabolic requirements. It is not until the oxygen pressure in the water has fallen to 50 mm. in *Planorbis*, and 7 mm. in *Chironomus*, that the oxygen combined with the hemoglobin is utilized. These forms are able to live in water poor in oxygen because their hemoglobin will take up oxygen at low pressures and transport it to the tissues. The condition appears to be similar in the earthworm (22) (42) and *Urechis* (61).

TABLE 6

Tension of Oxygen at Which Various Bloods Are Half Saturated under Approximately Physiological Conditions

SPECIES	TEMPERATURE	pH	CARBON DIOXIDE PRESSURE	OXYGEN TENSION OF HALF SATURATION
	°C.		mm. Hg	mm. Hg
Planorbis corneus (87)	20		0?	6.0
Arenicola (6)	20	7.3	7.3	1.8
Urechis caupo (61)	19	7.1	7.1	12.0
Siphunculus nudus (30a)	19		0–80	8.0*
Spirographis Spallanzanii (32a)	20	8.0		14.0†
Skate, *Raia ocellata* (20)	10	7.8	1.0	20.0
Mackerel, *Scomber scombrus* (65)	20		1.0	17.0
Sea robin, *Prionotus carolinus* (65)	20		1.0	16.0
Toadfish, *Opsanus tau* (65)	20		1.0	14.0
Cod (49)	15		0?	18.0
Plaice (49)	15		0?	10.0
Trout (49)	17		0?	11.0
Carp (80)	18		30	9.6
Carp (49)	17		0?	2–3
Pike (49)	17		0?	2–3
Eel (49)	17		0?	2–3
Amphiuma tridactyla (67)	26		43	28
Rana esculenta (87)	20		0?	10
Tortoise, *Pseudemys concinna* (70)	25		40	20–30
Snapping turtle, *Chelydra serpentina* (35)	20		42	22
Crocodile, *Crocodilus acutus* (19)	29	7.2		38
Pigeon (81)	42		40	50
Duck (81)	42		40	50
Goose (82)	42		50	37.5
Horse (35)	38	7.46	41	26
Man (35)	38	7.47	41	26
Sea lion, *Eumetopias stelleri* (31)	38		44	40
Porpoise, *Phocaena phocaena* (60)	38	7.25	46	31

* Blood contains hemerythrin.
† Blood contains chlorocruorin.

Krogh and Leitch (49) have extended this view to explain the pressures at which the blood of fishes combines with oxygen. Using the tension of oxygen at which the blood is half saturated as an index, they pointed out that in fresh-water fishes, such as the carp, eel, and pike, which are occasionally exposed to low oxygen pressures, the tension of half saturation is comparatively low as compared to marine fish, the cod and plaice, and such fresh-water fish as the trout, which are not normally exposed to very low oxygen tensions. They considered the properties of the blood in both cases to be specially adapted to the biological conditions.

In order that the data which have since accumulated may be considered from this standpoint Table 6 has been prepared. In it are entered the tensions of oxygen at which the bloods of the various species are half saturated under conditions of hydrogen ion concentration, carbon dioxide pressure, and temperature as near to those obtaining in the circulation of the living animal as can be, in view of the available data. The full oxygen dissociation curves for some of the bloods under these conditions are shown in Figs. 1 and 2. So far as the fishes are concerned, the newer data on the marine forms, the skate, mackerel, toadfish, and sea robin, fall within the limits set by the marine forms studied by Krogh and Leitch. *Arenicola* comes up to expectation in having a very low working tension. It is difficult to see, however, why *Urechis* should have a blood working at tensions as high as the trout, or the skate have a blood adapted to higher tensions, when temperature is taken into account, than the mackerel.

When the terrestrial vertebrates are considered, it is apparent that their bloods in general require higher oxygen tensions for half saturation. This fact is due in part no doubt to the higher temperatures at which these animals live, or maintain their body. At first sight one might expect the Amphibia and aquatic reptiles to have blood adapted to working at low tensions of oxygen, in a way similar to the carp and eel, yet this is clearly not the case. In order to properly weigh the data, it must be considered that the properties of the blood determine not only the conditions for the absorption of oxygen at the respiratory surface, but for its discharge from the blood into the tissues. Bloods with oxygen dissociation curves lying at low tensions favor the former and hinder the latter process. In each organism the optimal condition for the blood is a compromise in which these two processes are balanced. The factors determining the properties of the blood most favorable to the respiratory exchange are not only the tension of oxygen in the environment, but the structural and physiological properties of the respiratory organs and the vascular system as well as the general habits of the organism. The complex, and frequently antagonistic nature of these factors makes the teleological argument extremely dangerous, for it is easy to find some aspect of the problem for which the blood is well adapted, at the same time overlooking factors which may work the other way.

The tensions at which the bloods of the amphibians and terrestrial vertebrates combine with oxygen, when contrasted with the fishes, suggest an adaptation directed toward a more rapid and complete exchange of oxygen between the blood and the tissues. In the case of the aquatic reptiles, birds, and mammals, this may be an advantage in allowing the animal to utilize more completely the oxygen combined with the blood during periods when external respiration is interrupted by submergence. It is not clear how this would favor respiration in *Amphiuma*, which presumably absorbs oxygen from the water in which it is submerged. In the warm-blooded vertebrates, with their high metabolic rates, the high tension at which oxygen is held in the blood is of undoubted advantage in facilitating the rapid flow of oxygen from the blood to the tissues. In this way the properties of the blood of the birds (81) and the sea lion (31) are of advantage.

A different interpretation of these facts has recently been developed (59) from the consideration that the properties of the blood may be related to the characteristics of the atmosphere at the time when the various forms were evolved, rather than to the nature of their present-day environment. If the view postulated by certain geologists, that the quantity of oxygen in the atmosphere has increased since the earlier Paleozoic times, be accepted, it is not unreasonable to expect that the more recent group of animals may possess blood capable of taking advantage of this change. With this view the data here under review are in general accordance.

The relations of carbon dioxide to the oxygen equilibrium are also important and complicate the appraisal of any argument concerning the adaptation of the bloods to respiratory needs. It has been pointed out that the transport of carbon dioxide is dependent in large measure upon the buffer action of hemoglobin and that in most animals the character of the carbon dioxide dissociation curve is related to the quantity of hemoglobin in the blood and hence to its oxygen-carrying power. This is appropriate, for in general the need for transporting carbon dioxide is proportional to the oxygen consumption.

The pressure of carbon dioxide in the blood varies greatly in different animals, depending largely on whether the respiratory organs are gills or lungs. In the case of the fish the blood is exposed to very low tensions of carbon dioxide in traversing the gills, and consequently contains carbon dioxide at pressures which do not appear to exceed a few millimeters (20) (65). The hydrogen ion concentration of the fish blood is consequently lower than that usually observed in other vertebrates. This fact alone tends to cause the blood to combine with oxygen at low tensions and, together with the temperature of the water, accounts in large part for the generally low range of oxygen pressures at which fish blood functions. These factors do not, of course, explain the different characters of the blood of different fishes. Root has pointed out the high sensitivity of the hemoglobin of certain teleosts to carbon dioxide and has suggested that it is adjusted to function in an environment of low carbon dioxide tension, such as the gills offer. This point is of importance in evaluating the properties of the blood of the higher vertebrates. One might equally well say that the blood of fishes is ill adapted to functioning under the higher carbon dioxide pressures to which the blood of all pulmonate vertebrates is exposed and thus stress the adaptive character of the latter.

It has been pointed out that the carbon dioxide dissociation curve of the blood of a number of the lower vertebrates is peculiarly high and flat, owing to the large quantity of bicarbonate present in it. It is difficult to see what purpose is served by this special arrangement, as it can be of no advantage in buffering the blood against carbonic acid. It would, however, enable the animal to care for the accumulation of considerable quantities of non-volatile acid through the elimination of an equivalent quantity of carbon dioxide. It is noteworthy that the animals possessing blood with this character are all fresh-water vertebrates whose habitats or habits submit them from time to time to asphyxial conditions. If this generalization is valid one might expect a similar type of carbon dioxide dissociation curve in the crocodile. This is not the case, however, in the blood of the crocodile, of which the carbon dioxide dissociation curve is shown in Fig. 6. This blood contained a large quantity of lactic acid which may have decomposed the excess of bicarbonate. The high carbon dioxide capacities of the blood of the alligator observed by Hopping (38) when taken in connection with the accompanying oxygen capacities suggest that this animal also may have a carbon dioxide dissociation curve of the atypical form.

Arguments from the evolutionary and teleological viewpoints are equally treacherous and perhaps are not strictly pertinent to the scientific inquiry into the nature of the respiratory phenomena exhibited by the blood. It is safer and perhaps more valuable to inquire more closely into just what conditions characterize this function in the respiration of the living animal. There are sufficient data to define the conditions existing in the blood throughout the respiratory cycle in a number of animals and these are summarized in Table 7.

TABLE 7

Conditions Existing in the Blood in the Course of the Respiratory Cycle during Inactivity

SPECIES	URECHIS* (61)	SKATE (20)	SNAPPING TURTLE† (35)	DUCK‡ (81)	HORSE (35)	MAN (35)
Temperature, °C	19	10.4	20	42	37	37
Oxygen capacity, vol. per cent	4.50	6.00	6.40	17.0	21.4	20.5
Total O_2, vol. per cent:						
Arterial blood	4.1	5.90	5.82	16.6	20.0	19.4
Venous blood		1.98	2.12	6.6	13.5	14.1
Total O_2 transport		3.92	3.70	10.0	6.5	5.3
Saturation, per cent						
Arterial blood	97	93	95	95	98	96
Total CO_2, vol. per cent						
Arterial blood	8.8	7.70	96.43	47	44.9	48.2
Venous blood		10.84	99.39	55	50.3	52.0
Total CO_2 transport, vol. per cent		3.14	2.96	8	5.4	4.2
O_2 tension, mm. Hg						
Arterial blood	75	70	57.0	102	100	78
Venous blood		14	15.0	37	38	40
CO_2 tension, mm. Hg						
Arterial blood	7.2	1.3	38.5	54	41.6	40
Venous blood		2.6	45.2	65	49.2	45.4
CO_2 tension in respiratory organ	5.3	0				39
pH arterial serum	7.1	7.82	7.60		7.43	7.455
pH on becoming venous		−0.15	−0.06		−0.03	−0.026

* Conditions in coelomic fluid entered as arterial blood.

† Blood from lungs entered as arterial blood; blood from tissues entered as venous blood. For conditions in systemic arteries, see Henderson (35).

‡ Mean values from author's data.

In *Urechis*, a large worm of sedentary habit, there is no vascular system. The coelomic fluid, which functions as blood, has a relatively large volume and, although the erythrocytes are not numerous nor rich in hemoglobin, the total oxygen capacity is sufficient to last the animal for about an hour. One cannot distinguish between arterial and venous blood or properly speak of a circulation. The coelomic fluid is merely churned about through peristaltic movements of the body wall and hind gut, successive portions of it being brought into contact with the active tissues or with the wall of the hind gut which forms the respiratory surface. The latter organ is "ventilated" with fresh water with sufficient regularity to keep more than a third of the oxygen within it from being absorbed. This gives sufficient oxygen pressure to saturate the blood almost completely and since only one-sixtieth of the blood's oxygen is used each minute, the blood as a whole remains so saturated. The coelomic fluid of *Urechis* serves as a reservoir for oxygen, keeping each tissue surrounded by an excessive supply of this gas, rather than as a means of bringing a constant stream of oxygen to the active parts. Carbon dioxide accumulates to a pressure of about five millimeters in the hind gut and consequently carbon dioxide is retained in the blood, but not at pressures greater than 7 millimeters. The coelomic fluid is unbuffered save for hemoglobin and consequently becomes more acid than sea water or the blood of higher forms but does not exceed neutrality.

In the skate anatomical considerations produce a situation in marked contrast. With the presence of a closed circulatory system the blood is concerned solely with *transport* of oxygen. The hemoglobin is well oxygenated in the gills, and in traversing the tissue capillaries gives up two-thirds of its oxygen content. This utilization, large when contrasted with the higher forms, implies a corresponding economy in the rate at which the blood is circulated and correlates with a circulatory system in which the blood must traverse two capillary systems in each circuit. Because of the high degree to which the oxygen is utilized, the pressure of oxygen in the venous blood, and presumably the mean pressure in the capillaries, is low. The pressure-head driving oxygen from blood to tissues must be correspondingly low, but this is tolerated because of the generally low metabolic rate of this inactive fish. In passing the gills the blood is exposed to water of negligible carbon dioxide content. The pressure of carbon dioxide in the blood and its carbon dioxide content are accordingly low and the pH value high. Corresponding to the large utilization of the oxygen in each increment of blood, there is an equally great carbon dioxide transport, relative to the total buffering capacity. The acid base equilibrium is shifted consequently as the arterial blood becomes venous and an unusually large change in pH of the blood results.

The snapping turtle resembles the skate in the fact that the utilization of oxygen is relatively great. In this form also the circulatory mechanism is spared at the expense of the oxygen-transporting power of the blood. The circulatory system is handicapped by the mixing of pulmonary and venous blood in the ventricle, so that the blood in the systemic circulation is not fully oxygenated and has characteristics midway between those listed as "arterial" and "venous" in Table 7. This fact must be important in necessitating the high utilization of the oxygen transport. The turtle's blood is well oxygenated when leaving the lung, although the pressure of oxygen in it is relatively low owing to the incompleteness in the ventilation of the lung. Because of the latter fact and the high degree to which the oxygen in the blood is utilized, the mean pressure of oxygen in the capillaries must be relatively low. The principal difference distinguishing the turtle from the fish consists in the high carbon dioxide tension in both arterial and venous blood, which is a reflection of the carbon dioxide accumulated in the lung and the extraordinarily great total carbon dioxide content due to the atypical character of the carbon dioxide dissociation curve. These factors interact to give a pH value to the arterial blood which is higher than that found in the mammals though not so high as in the blood of the fish.

In the blood of the warm-blooded vertebrates the characteristics of the changes in the respiratory cycle reflect an organization suited to more intense metabolic activity. The oxygen capacity of the blood is much higher and the vascular system of these forms is suited to circulating the blood with greater rapidity and under higher pressure. In spite of the higher metabolic rates of the mammals, sufficient oxygen is supplied to the tissue without the utilization of such a large fraction of the oxygen contained in the blood, a possibility realized by virtue of the higher oxygen capacity and the more rapid rate of circulation. The pressure of oxygen in the venous blood and consequently the mean pressure existing in the capillaries, is maintained at a higher level than that observed in the cold-blooded forms. In this way a high pressure-head is provided for the rapid diffusion of oxygen from the blood to the tissues. The low degree of utilization of the oxygen present in the blood also leaves a larger margin which may be drawn on by the tissues during increased activity. The pressure of carbon dioxide in the blood is high since the ventilation of the mammalian lung is not sufficiently rapid to prevent the considerable accumulation of carbon dioxide in the alveolar air. The blood contains in consequence a high total carbon dioxide content, but as most of this is in the form of bicarbonate, it still retains a distinctly alkaline reaction. It is noteworthy that in the mammals the change in the pH in the blood passing from artery to vein is small compared to the lower forms, reflecting the higher development of the mechanisms for preserving the constancy of its physicochemical state.

The general picture presented by the blood of the duck differs from that observed in the mammals chiefly in the greater extent to which the oxygen circulating in the blood through the capillaries is utilized. It is difficult to be sure that this difference is not due to disturbances in the circulation or respiration incident to the collection of blood samples for analysis, since this was done, in the case of the data recorded for the duck, under anesthesia. However, the shape of the oxygen dissociation curve in the duck is such as to favor a high degree of utilization without corresponding loss in mean capillary oxygen pressure. It is not impossible that the high activities and metabolic rates of birds are facilitated by this character of the oxygen dissociation curve, enabling a greater quantity of oxygen to be transported by the blood and used without increasing the work which the heart must do in circulating the blood.

These cases make it clear that in the evolution of animals a wide variety has arisen in the quantitative aspects of the functioning of the blood in respiration. They depend on the specific properties as well as on the relative proportions of the constituents of the blood, and on the relations which exist between the activity of the animal, the nature of its environment, and the structure and function of its circulatory and respiratory mechanisms. On the whole, the picture is one of a system developing simultaneously in its various parts toward greater efficiency in supplying the requisites for activity, for terrestrial life, and for stability of function in the face of varying need.

In compiling the data presented in this paper it has been necessary frequently to change the units of measurement and carry out other minor mathematical transformations on the figures presented by the authors of the original work. For such changes the writer assumes full responsibility. References should consequently be taken as indicating the source from which the original information was obtained. In the citation of literature no attempt has been made at historical completeness and no attention has been paid to questions of priority as these matters are sufficiently presented in certain of the general papers referred to (1) (4) (5) (16) (30) (35) (64) (86). It has been the endeavor to draw attention rather to the principal recent investigations which contribute to the subject.

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Contribution No. 14

SOME PHASES OF MODERN DEEP-SEA OCEANOGRAPHY

WITH A DESCRIPTION OF SOME OF THE EQUIPMENT AND METHODS OF THE NEWLY FORMED WOODS HOLE OCEANOGRAPHIC INSTITUTION

BY

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SOME PHASES OF MODERN DEEP-SEA OCEANOGRAPHY

WITH A DESCRIPTION OF SOME OF THE EQUIPMENT AND METHODS OF THE NEWLY FORMED WOODS HOLE OCEANOGRAPHIC INSTITUTION[1]

By C. O'D. Iselin, ii

[With 4 plates]

Until recent years physical oceanography was too frequently considered a branch of science having little importance except to those interested in the ocean itself. This feeling perhaps resulted from the fact that on the earlier expeditions the main emphasis was placed on marine biology, and physical observations were first made mainly with the purpose of exploring the environment of marine plants and animals. Later there developed the necessity of studying the ocean currents and their influence in the transportation of sea life. As a result the physical oceanographer came to be thought of as one who charted the various physical characteristics of the ocean and who had as his ultimate goal the understanding of oceanic circulation.

At the present time, however, it is becoming increasingly evident that several other branches of science must turn to the sea, and therefore to the oceanographers, for help, for only in oceanography can be found the men and equipment for the investigation of the ocean and the problems related to it. Let us briefly consider some examples in which the oceanographer can do important work outside the original conception of his field.

In the first place, geology is naturally much concerned with the ocean basins. Have they been permanent features on the earth's surface, or in the past has there been dry land where now the chart shows one or more thousand fathoms of water? Convincing proof that will settle this problem one way or another can perhaps be brought to light by the oceanographer. A careful study of marine deposits might point to former land bridges between the continents in such a way that they could be accurately located and even dated, much to the satisfaction of all students of the geographic distribution of modern plants and animals, as well as to geologists. Perhaps a less spectacular problem is that of the formation of continental shelves. Again there is reason to believe that the question can best be settled by a careful study of bottom deposits. Thus oceanographic institutions, having developed a technique for bringing up deep-sea mud in the course of exploring the oceans, are now realizing more fully the wide geologic importance of this type of work. With proper equipment much can be learned from the ocean bottom which will extend geologic knowledge out beyond the beach line and perhaps open up a sounder theory for the formation of the earth's surface.

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[1] Contribution No. 14, of the Woods Hole Oceanographic Institution.

In the same way, but perhaps not to the same extent, meteorological investigations have been largely confined to the air above the continents. Not only is there much meteorological research work to be done at sea, but this science is closely bound up with oceanography. For example, how much influence have fluctuations in the ocean currents on variations in climate and vice versa? From a more technical standpoint, students in dynamic meteorology and dynamic oceanography now see more clearly how similar is the principle of the circulation of the atmosphere to that of the ocean. Thus we can expect in the future a closer relationship between the physical oceanographer and other geophysical students, for many of their problems are interrelated.

On the other hand, the scope and importance of marine biological work has been more generally recognized and in certain lines, for example, fisheries investigations, has developed relatively fast. The many marine laboratories of the world are good evidence that physiologists have realized the importance of studying life in its most natural environment. Hand in hand with the development of marine biology has gone the study of the chemistry of sea water and such questions as the penetration of light below the sea surface and its influence on the life in the upper water layers.

Thus at the present time, oceanography has passed the stage of a science in which the collection and tabulation of facts is considered the main aim, and it is now evident that much productive research of wide interest can be carried out at sea. The reader perhaps now realizes that an oceanographic institution must have on its staff men of wide training and experience in science as well as the specialists in the more restricted phases of oceanography.

The investigation of the ocean naturally divides itself into deep-water problems and shallow-water problems. The well-known fact that most continental land masses are surrounded by a broad, relatively shallow shelf of water less than 100 fathoms deep and that the ocean basins are uniformly twenty or thirty times as deep, serves to emphasize this distinction. Investigations along the coast and even out as far as the edge of the continental shelf can be carried out from almost any boat and with quite inexpensive equipment. Deep-sea oceanography, on the other hand, is an expensive undertaking requiring a strong, able vessel and elaborate winches and instruments. For these reasons there are a number of scientific institutions actively engaged in studies of the shallow-water areas of the ocean, but relatively few can conduct researches outside the hundred-fathom curve. In several ways this state of affairs has been unfortunate in the development of some phases of oceanography, because the shallow waters over the continental shelves are usually much affected by the circulation of the waters of the ocean basins, while tidal and other influences often obscure the picture to such an extent that isolated problems can not be easily settled.

On the other hand, the shallow-water areas are, of course, the seat of the world's fisheries and for that reason merit governmental study. But even in the case of fisheries investigations, the oceanic waters can not be ignored, because it has been shown that the sudden failures of a fishing ground are sometimes caused by movements of the oceanic waters which periodically flood in over the banks, changing the temperature of the bottom water and driving off the fish. In other words, the investigation of the sea has progressed largely from the shore outward, while it is now evident that there would have been some advantage to oceanography as a whole if it had been possible to put more effort into deep-sea investigations during the early stages of the science. It is perhaps not an exaggeration to say that the heart of most oceanographic problems is to be found in the deep ocean beyond the limits of the continental shelves.

The fact that there has recently been established in the United States an institution largely devoted to deep-sea oceanography is therefore of considerable interest. The Woods Hole Oceanographic Institution, with its headquarters on Cape Cod, is ideally situated for the investigation of the North Atlantic Ocean. The institution is now a going concern with adequate money to support a well-equipped laboratory at Woods Hole, Mass., and a specially designed research ship. If, in the succeeding pages, the reader finds that we have stressed the equipment and methods of this institution unduly, it is because it was founded to counteract the situation in oceanography outlined above and in many ways, we believe, holds a unique position in the world to-day.

The main distinction between an oceanographer and other men of science is that the oceanographer goes to sea. His ship is the all-important part of his scientific equipment for he must be able to make observations in all kinds of weather and at great depths. Not only must he often remain far out at sea for months at a time, but also he must be ready to take an active part in securing his observations.

No matter how well trained the research ship's crew may be, the scientist must thoroughly understand the difficulties and limitations of the work, or he will not be able to evaluate the results properly or suggest practical programs for scientific cruises.

Many types of vessels can be used for oceanography with good results. Fishing craft of various types have proved very satisfactory. The main points are that the vessel should be entirely seaworthy and able to remain at sea longer than ordinary commercial usage requires. For the investigator of the chemistry of sea water especially, the steadier the ship, the more accurate will be his work. But to gain steadiness by using a large ship is often a disadvantage. If the boat is too large, besides being expensive to operate, the scientists are not close enough to the water to handle easily much of their equipment. One solution is to use a sailing vessel, because the sails tend to prevent the boat from rolling excessively in a rough sea. In oceanography, then, we find one instance, at least, where the sailing ship can hold out successfully against the inroads of steam.

One of the most modern examples of a scientific research ship is the *Atlantis* operated by the Woods Hole Oceanographic Institution. She was built especially for the work, after considerable thought, and embodies the experience of many men familiar with carrying out scientific work at sea. The result is a boat that is very satisfactory and at least represents one solution to the problem. She is an auxiliary, steel ketch of about 460 tons displacement, and carries 7,200 square feet of canvas. Her general dimensions are: Length on deck, 142 feet; beam, 29 feet; and draft, 17 feet. She is powered with a 280-horsepower Diesel engine, which besides propelling the ship at a speed of 9 knots in calm weather, supplies through a dynamo the power for the heavy trawl winch. A much smaller Diesel engine generates the power for light, ventilation, and refrigeration, as well as for the hydrographic winch. Perhaps the most specialized piece of equipment is the trawl winch, located, because of its great weight, in the lower hold. It carries 5,000 fathoms of special steel cable of ½-inch diameter, with a breaking strain of about 12 tons. The hydrographic winch, used mainly for lighter work such as securing deep-sea temperatures and water samples, carries a similar length of much lighter wire and is located on deck. Both winches are electrically driven and fitted with automatic devices for guiding the wire smoothly on the drums.

The *Atlantis* accommodations include cabins aft for a scientific staff of five, and amidships two laboratories, one opening out to the deck where the rough work is done and another directly below it where the chemical analyses are carried out and where the microbiologist can examine the catch of his silk net without being disturbed. The permanent crew, exclusive of the scientific staff, numbers 17 men. They have comfortable quarters, and there is ample storage space below decks. The vessel is a smart sailer and an excellent sea boat. Her rig, though small for the size of the hull, is efficient and ideally adapted for heaving to, which is most important for this type of work.

The primary instrument of the physical oceanographer is the deep-sea reversing thermometer. These are now sent down in pairs, one open to the pressure of the water and the other inclosed in a heavy glass case. The significance of this we will see presently. Both the protected and the unprotected thermometers are of the same construction. On being turned over the column of mercury breaks off at a constriction in the capillary and the temperature is read as the length of this detached thread of mercury. Thus the reading can not be changed on the long haul to the surface by passing through the much warmer surface layers. The deep-sea thermometers are now very accurate and can record the temperature to a hundredth part of a degree centigrade. The pairs of thermometers are sent down in frames which are mounted on the side of instruments known as water bottles. These are fastened to the wire cable at suitable intervals and lowered over the ship's side. A small weight, or messenger, is then slid down the wire, which, on striking the uppermost instrument, closes the openings of the water bottle, reverses the thermometers, and releases another messenger which slides on down the wire and repeats the operation with the next instrument. Thus a series of 10 or more water samples and pairs of thermometer readings can be secured at one lowering. In deep water it may take several such lowerings, each successively deeper, to constitute a station.

Although the thermometers and water bottles have been in use for many years and have gradually become much improved in design, there was still one grave inaccuracy which has only recently been eliminated. The depth of each observation was formerly recorded as the length of wire, measured on a wheel of known circumference, from the sea surface to each water bottle. However, in general the wire did not remain vertical in the water. The angle the wire took depended on the relative motions of the ship, the surface layers, and the deep, nearly motionless water masses. In other words, the wire would not only enter the water at an angle, but probably formed an **S**-shaped curve of unknown extent, so that the depths of all observations was problematical. Since one of the purposes of temperature and salinity observations is to assist in the study of ocean currents—and in regions of currents the trouble of large wire angles (often as much as 40° from the vertical) is at a maximum—it was a great advance when the use of unprotected thermometers became general a few years ago. This method really measures the pressure existing at each instrument of a given series and from these the depths are calculated. The pressure of the water compresses the glass of the unprotected thermometer and makes it read higher than the protected thermometer with which it is paired. The difference in reading between the two instruments can then be translated into depth and with a surprising degree of accuracy. Not only are reliable depths now secured for the temperature observations taken from a modern research ship, but of course these same depths are good for the samples brought up by the water bottles. The water samples are of sufficient volume so that besides salinity, several other chemical factors can be determined. In fact, all physical observations made with this apparatus are now on a comparatively accurate basis.

The primary collecting instrument of the marine biologist is, of course, a net of one sort or another. Many different types are employed, depending on what type of animals or plants are sought and on whether the collections are to be obtained from the bottom, the surface, or intermediate depths. Of late years, attention (formerly directed chiefly to the bottom fauna) has been largely centered on the drifting community of animals and plants—the so-called "plankton"—which are captured as a rule with some form of tow net. Tows made deep down in the water have, until recently, been confused by the same inaccuracy as have the routine physical observations, namely, the uncertainty as to the precise depths from which the captured specimens came. Not only was the maximum depth reached by the net uncertain but also the animals might have gotten in on the way down or on the way up. This last source of error is especially serious in the case of deep-sea tows, for with these the time involved in lowering and raising the net may be several times as long as the horizontal tow itself. Even for plankton work near the surface, it has been difficult to know at just what depth the net had been fishing or to do accurate quantitative work with more than one net at a time. Improvement in the technique of tow-net work has come gradually and is perhaps not yet at a satisfactory stage of development.

One of the first steps was the perfecting of nets which can be sent down closed, opened during the tow, and closed again before being hauled to the surface. Another improvement has been the use of a kind of bucket at the back end of the net which protects the catch from being rubbed too much against the netting during the long haul to the surface. As many deep-sea animals are ruined as specimens through this cause as through the release in pressure on nearing the surface. This destructive release in pressure, which at first destroyed nearly all specimens of deep-sea fish by causing gas bubbles to form in the tissue, can now be fairly well prevented by using much the same methods as employed in raising a diver.

Recently on the *Atlantis* it has been found possible to make as many as five simultaneous tows with closing nets and know the exact depth of each catch. In this method the wire is so heavily weighted that it remains practically vertical during the tow and the nets are fastened to it one below the other at known intervals. A system of messengers sent down the wire operate the opening and closing devices of the nets. Not only are the opening and closing devices extremely satisfactory but also a further refinement has resulted. Since the frames of the nets are fastened to the wire rigidly, there is no bridle in front of the opening to scare away the animals in the net's path. With large deep-sea nets designed for catching more active animals such refinements are not yet in practice. In fact, even though the net used may be of the closing type, the depth at which the catch is made usually becomes somewhat problematical if the towing wire is at any considerable angle from the vertical. There has been some experiment with pressure instruments which should record the depth of the net at all times during the tow, but there are still many difficulties which must be met. The technique of deep-sea tow-net work is further complicated by the fact that the nets used to-day do not stand much hard usage and soon develop holes and tears which always try the patience of the oceanographer. It is a matter of time and money before biological work at sea can be conducted with satisfactory accuracy.

The study of bottom samples taken from the floor of the ocean has always been productive and has aroused considerable interest among geologists as well as oceanographers. The recent developments in this field have also much improved the accuracy and use of the observations. The old-fashioned piano-wire sounding machine can now be replaced by the accurate and almost automatic sonic method, so that the exact depth of the water can be found at all times. Formerly it took several hours to make each sounding and by the time the wire was hauled back aboard, the vessel could have drifted into water having a different depth. For all bottom sample work, the operation is made much easier by knowing the depth in advance. Otherwise several hundred fathoms extra wire may be run out before the observer is sure his sampling device has reached bottom. With any sampler more complicated than the ordinary sounding tube this may be a great disadvantage. The extra cable coiled on the bottom may kink and the instrument will probably be lost when strain is again put on the wire.

It is a great advantage to investigators to have the bottom sample come up in a water-tight condition so that none of the fine washings are lost on the long haul to the surface. To meet these requirements much attention has been devoted, of late, to devising improvements on the simple " snapper " and other devices which have long been used. For example, various types of valves have been tried that will prevent the mud from washing out of the sounding tube. Other more modern samplers are designed to secure long cores of the bottom material. With the present equipment, samples up to 3 or 4 feet in length have been brought up, and these have shown that the deposits on the ocean floor are often stratified. It is through the study of such material that eventually the problem of whether or not the bottom of the present deep ocean has ever been above sea-level will probably be settled. Still another type of sampler has been gradually evolved that will take either sand or mud from the region over the continental shelf. Another type, mainly used in shallow water, brings up a given area of the bottom which can then be studied for the plant and animal life and their relations as a feeding ground of commercial fish.

We have perhaps described enough of the modern oceanographic equipment to show the reader that not only is the design of all gear being gradually improved, but that the technique of securing good observations from a small ship can not be easy even in favorable weather. It is this mechanical or engineering side of oceanography that has attracted a good many men to the field. It often seems to the harassed investigator that the sea hides some monster which is most antagonistic to having his realm explored. Unforeseen things are constantly hampering the work of each oceanographic expedition. For example, there are several kinds of marine animals which become wound around the hydrographic wire and stop the messengers. Often a piece of apparatus comes up which has not worked because the messenger never reached it, and this after hours of waiting while miles of cable were unwound and rewound on the winch. If the submarine " devils " are not interfering with the work, the " devils " of stormy weather are very apt to seize the opportunity to persecute the sleepy oceanographer who has perhaps been struggling for hours to complete a series of observations. In wintertime it is a real fight to go to sea and to return home with any of the secrets of the sea safely recorded in the scientific log book. Thus storms and salt water must be combated continually; and although most sensible people very wisely stay ashore, the work at sea holds a real fascination through its difficulties and discomforts, to a small but enthusiastic group of men working in the various oceanographic fields.

In order that those unfamiliar with deep-sea oceanographic work may get some idea of the type of problems now being studied, we will describe presently the investigations made by the *Atlantis* during the past year. Since these have been largely in the field of physical oceanography it will be necessary first to point out a few of the features of the circulation of the North Atlantic, bearing in mind that, although we will be discussing the North Atlantic, the same problems exist in the other oceans, as they all work in the same manner. Therefore, discoveries in any ocean can usually be applied to the others. In other words, it is not necessary to sail very great distances to do important oceanographic research.

The current system of the North Atlantic basin, as in the case of the other oceans, is partly convectional in nature and partly wind-driven. Since the water is heated near the equator and cooled in the north, a current system is naturally set up owing to the distribution of density resulting from the thermal inequalities. On the other hand, the evaporation at the sea surface is probably on the whole greater than the precipitation in the southern half of the ocean, and the reverse in the north. Through this cause, the surface water is made relatively heavy in the south and thus the distribution of density due to temperature is partly counteracted and the convectional circulation, much dependent on temperature, somewhat retarded. It is thought that the Gulf Stream system is largely a manifestation of this need for thermal transfer from south to north.

In the southern North Atlantic the water movements are probably dominated by the trade winds which blow the surface waters westward towards the islands of the West Indies, so that the water level in the Caribbean Sea and the Gulf of Mexico may be somewhat higher than farther north. At any rate, the result is a swift current through the Straits of Florida and the beginning of the Gulf Stream, which carries some of the warm southern water northward to the Grand Banks and then eastward toward Europe. But the forces which maintain the eastward drift in the northern latitudes are probably only partly convectional in nature, for in these latitudes the prevailing westerly winds undoubtedly exert a strong influence. In this manner a huge clockwise eddy is maintained in the North Atlantic.

Owing to the fact that the waters of the ocean are usually arranged in stable layers, and that Archimedian forces tend to keep these layers horizontal, unless disturbed by some outside force, the character of such a current as the Gulf Stream is surprising. Through the effect of the earth's rotation the normally horizontal water layers are sloped across the path of any current so that the lighter water lies deeper on the right side (in the northern hemisphere). In the case of the Gulf Stream, this means that the warm water must lie deeper on the southeastern side. In other words, half of the current consists of relatively cold water and half of relatively warm water, because the isotherms slope sharply across its path. In the case of the 10° isotherm, for example, this means a slope of from 200 meters on the western side of the stream to 800 meters on the offshore edge, the current being about 50 miles wide off New York. The Gulf Stream then is not a river of warm water flowing through colder seas, but the boundary between a body of relatively cold water and the mass of warm, central, Atlantic water. It follows then that the Gulf Stream, by which we mean the whole of the convectional current along the eastern North American seaboard and not just the surface layer, does not transport the warmest water northeastward. Even at the surface, the warmest water is not over the stream unless displaced by easterly winds. From a technical point of view, it is wrong to think of the Gulf Stream as being a river of warm water, although it is the existence of warm water in the southern North Atlantic which is its cause. The current, because of the earth's rotation and the stable arrangement of the water layers, only accomplishes what it sets out to do in a most inefficient manner.

But besides this horizontal circulation, at the same time there exists much slower vertical movements. Some of the cold, relatively heavy northern water sinks and gradually moves southward along the bottom, only to rise again near the equator. That these two systems exist can be clearly and easily demonstrated from the observations already recorded, and some progress has been made by studying the pressure field of the ocean in much the same way as is done in meteorology. The region south and east of Bermuda, known as the Sargasso Sea, corresponds to a permanent high-pressure area on a weather map. Such southward-moving tongues of cold water as the Labrador current can be compared to the polar fronts of the meteorologists. But sea water, in contrast to air, is nearly incompressible and is usually found to be well stratified and therefore quite stable. Moreover, it is out of the question for oceanographers to secure any such instantaneous picture of the pressure field as in a weather map and besides it is not known to what extent the ocean circulation is wind driven.

Thus it is an unfortunate fact that as we look more closely into the ocean currents and attempt to find reasons for their paths and characteristics, we must admit that this phase of oceanography is at a most awkward stage. The existing observations are too few to explain more than the broadest picture, and as each expedition brings back modern data, that is, sections made up of closely spaced stations, it is evident that many of the early ideas are unsound. Since it would be impracticable for any one institution to attempt a complete survey of the North Atlantic, all existing observations must be combined to show the general scheme of circulation and test cases must be studied to explain the peculiarities.

At the present time, among the foremost problems in ocean circulation are the following: (1) The relative importance of wind and convectional forces; (2) the question of whether ocean currents flow steadily and in continuous paths or spasmodically; (3) the effect of seasonal changes in surface temperature. As an initial approach to the first problem there is a need for quantitative data. What force does a wind exert at the sea surface? The question has been studied by the mathematician, Ekman, and the theory of his famous spiral is well known. It has been suggested that if the wind drives the surface layers of the ocean they in turn must retard the lower strata of air. Ekman's spiral should exist in both the ocean current set-up and in a reversed direction in the lower layers of the atmosphere. Since it is more easily measured from the meteorological point of view through pilot-balloon observations than in the ocean, it may be possible for students of aerodynamics to secure for oceanographers actual values of the force exerted at the sea surface by the wind.

The second problem, concerning the steadiness of ocean currents, is perhaps too technical to discuss here since it involves the usefulness of Bjerknes's equations. His theory for calculating the velocity of currents by the slope of the surfaces of equal density demands that the circulation has assumed a steady character. There is some reason to believe that ocean currents are usually either slowing up or increasing their velocity and often break down altogether. The immediate question before physical oceanographers is how far it is advisable to use for dynamic calculations stations from different expeditions or even from the same expedition, but made over a period of a month or more. It may be that in certain regions the ocean circulation is disturbed by eddies corresponding to the extra-tropical cyclones of meteorology, in which case nothing but simultaneous stations will give a true picture of the circulation. In the case of the northern areas of the Atlantic, the third problem is immediately brought up. Are the seasonal temperature changes in the surface layers powerful enough to alter the underlying dynamic forces?

There are many more perplexing questions having to do with circulation which are still unsettled, but the above is perhaps sufficient to show that the understanding of oceanic circulation is still in a very elementary stage in spite of what one might think from examining the very considerable literature on the subject, unfortunately mainly based on insufficient data. In other words, the great need at present is for observations especially planned to bring out particular points rather than for stations scattered over wide areas. The crying need is for proof.

Now it may seem to the reader that oceanic circulation is a relatively unimportant scientific problem. It is true that for the purposes of navigation we now have sufficient knowledge of the main currents and that for such services as the ice patrol the existing methods give good results as applied to small areas. The reason why we have here stressed circulation is that almost every problem in deep-sea oceanography is somehow linked to it. The marine biologist can not adequately account for the distribution of life in the sea without a good knowledge of the water movements. The quantity of life in the sea is also linked to the current system since it depends on the supply of certain chemicals in the water. These essential chemicals are found in greatest abundance near the shore because they are brought to the sea by the rivers. When in a given area they are used up more quickly than the currents can carry them out from the shore, the population will die out. The sea is the original home of life as we know it on earth. For countless ages the evolution of marine forms must have been greatly influenced by the supply of these vital chemicals. If it were not for the circulation of the oceans, no matter how slow, large areas of it would be completely barren. The important part which the ocean currents are now playing (and have played in the past) in the climate of the world is more generally appreciated.

Anyone who has had experience in research work knows only too well how one thing leads to another. An investigator no sooner gets started on a problem than he finds that he can not progress until he has settled another problem, which only develops after he has begun work on the first. In oceanography the difficulty is increased by the fact that such vast distances have to be covered and it is usually impossible to repeat the work. It almost seems that it requires the experience of a preliminary cruise to know how to attack any given problem. For this reason anyone planning a program for scientific work at sea would do well not to attempt too much on one cruise. There is little hope of finding an important problem that can be settled by one expedition. Often it is the development of a technique for handling new apparatus which is the main result of an elaborately planned cruise. There is a vast supply of oceanographic problems, but little is known about methods for settling them.

With the formation of the Woods Hole Oceanographic Institution and the building of the *Atlantis* those interested realized only too well the difficulties of laying out a program. No longer was it a question of planning for an isolated expedition which must yield a maximum of results in a given length of time. Rather, some policy had to be adopted which would tie together the very scattered information already in existence and at the same time prepare for future work that was bound to progress slowly.

The accompanying diagram shows the sections run by the *Atlantis* during the first 10 months of her existence. These sections consist of about 250 stations along lines totaling almost 10,700 miles in length. About two-thirds of the stations lie in deep water. Observations have been made always to 3,000 meters, when the depths of the water allowed, and at frequent intervals to the bottom.

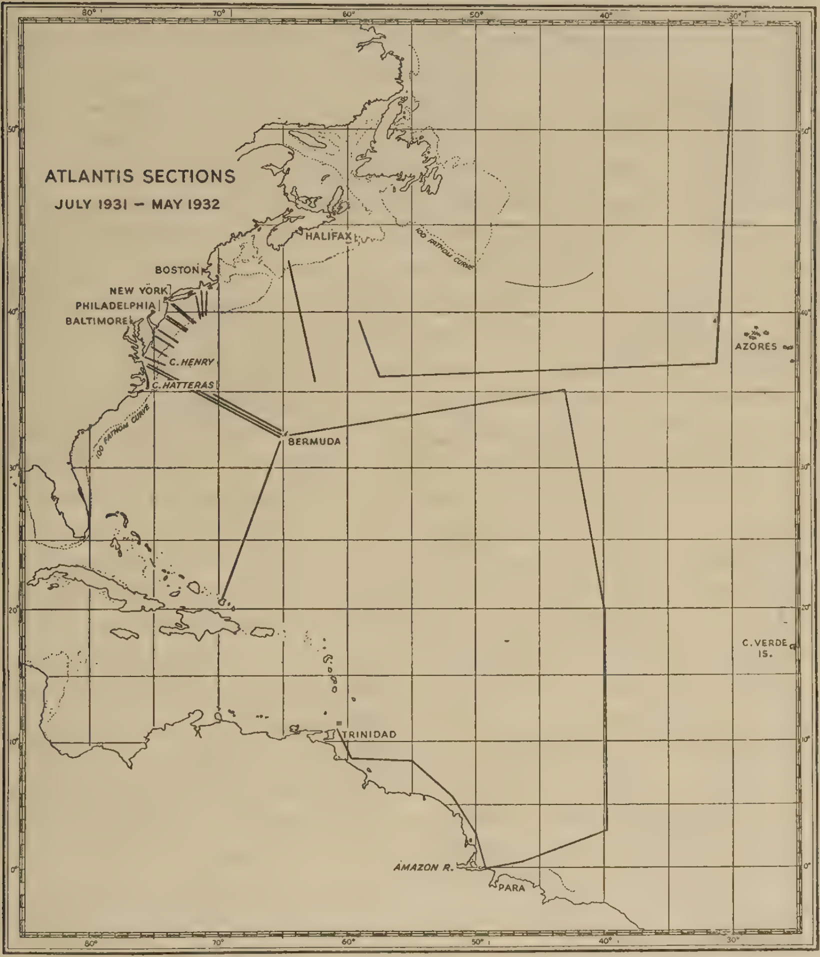

ATLANTIS SECTIONS
JULY 1931 – MAY 1932
HALIFAX
BOSTON
NEW YORK
PHILADELPHIA
BALTIMORE
C. HENRY
C. HATTERAS
100 FATHOM CURVE
BERMUDA
AZORES
C. VERDE IS.
TRINIDAD
AMAZON R.
PARA
80°
70°
60°
50°
40°
30°
50°
40°
30°
20°
10°
0°

FIGURE 1.—*Atlantis* sections, July, 1931–May, 1932

The physical program of the institution centers around the two sections out to Bermuda. It is hoped that it will be possible to repeat these sections quarterly. They should provide information concerning fluctuations in velocity and in volume of the Gulf Stream. They should show whether or not its path varies with the season and from year to year. Since on these sections the stations have been spaced at from 15 to 25 mile intervals, they should give reliable data on the internal arrangement of the water layers in what is perhaps a purely convectional current. The cruise from Nova Scotia to Bermuda and back to Chesapeake Bay can usually be made in under two weeks, so that the sections come very close to satisfying the ideal of giving an instantaneous picture.

Although this work is perhaps mainly of interest because it will supply detailed information on the Gulf Stream, the greater part of the sections lie in the relatively motionless mass of the central North Atlantic water. In this region between the Gulf Stream and Bermuda the stations will be most helpful in the study of such questions as internal boundary waves, the mixing action of storm waves, and other questions important over the whole ocean rather than applying to currents. Between the Gulf Stream and the edge of the continental shelf there is also another interesting band of water which is relatively cold at all depths when compared to the water just to the south and eastward. Yet it is not at all certain that this strip of water, which can be thought of as keeping the Gulf Stream away from northern Atlantic coasts, has a northern origin.

Besides these Bermuda sections the *Atlantis* has run two long north and south profiles in mid-Atlantic. The more northerly line of stations follows longitude 30° W. and crosses the North Atlantic Drift, as the continuation of the Gulf Stream is sometimes called. Before this section was run (July, 1931) there was evidence that the Gulf Stream split into at least three branches after leaving the region of the Grand Banks and that the prevailing westerly winds in this part of the ocean played an important part in the easterly movement of the surface waters. The *Atlantis* section of closely spaced stations should furnish important data concerning the water movements in this part of the ocean and at the same time definitely establish the branching nature of the continuation of the Gulf Stream.

The southern of these two profiles (February, 1932) follows longitude 42° W. and extends from the horse latitudes to the Equator. It therefore crosses the northern equatorial, or trade-wind, current. In theory, this section should show up the nature of a pure wind current besides giving the volume and velocity of the westerly drift across the southern part of the North Atlantic. It was hoped that the arrangement of the water layers in this type of current would be strikingly different from those of the convectional current, so that it would be possible to decide roughly in other profiles how much of the movement was due to wind and how much to convectional forces. A preliminary examination of the observations has indicated that this was perhaps too much to hope for, yet the section will be most important when the time comes to establish in detail the water movements of the North Atlantic.

In the course of running these important sections, opportunity was found for examining several more special regions. In the first place, a section was made off the Amazon River (March, 1932) showing the transition from oceanic conditions to those of fresh water. This section will be mainly of interest from the chemical point of view. In the second place, a good section from the West Indies out to Bermuda was made (April, 1932) to show up the nature of the Antilles current. This is the current which is supposed to carry southern water northward outside the islands and to join with the Gulf Stream near the Straits of Florida. Finally several short sections have been run across the coastal waters between Cape Cod and Cape Hatteras, on the eastern seaboard of the United States. This work was in connection with a study of the coastal conditions throughout the year begun by the United States Bureau of Fisheries.

So much for the routine temperature and salinity observations made in deep water during the first year of the Woods Hole Oceanographic Institution. It must be remembered that in the course of collecting this huge number of water samples, the opportunity was not lost to examine them for oxygen content, P_h, P_2O_5, and PO_4. Therefore, the work has a most important chemical aspect besides giving data for the study of ocean currents.

In the field of marine meteorology, the work carried out on board the *Atlantis* has been mainly on two problems. Considerable data have accumulated that are being used to show more accurately the relationship between the rate of evaporation at the sea surface and the wind velocity, as well as its dependence on the stability of the lower layer of the atmosphere. About 100 pilot-balloon ascents have been observed with a theodolite over a large area of the Atlantic with a view of finding the frictional force exerted by the wind on the ocean surface. As is usually the case with scientific work at sea, it has taken considerable time to develop a satisfactory technique in making these observations.

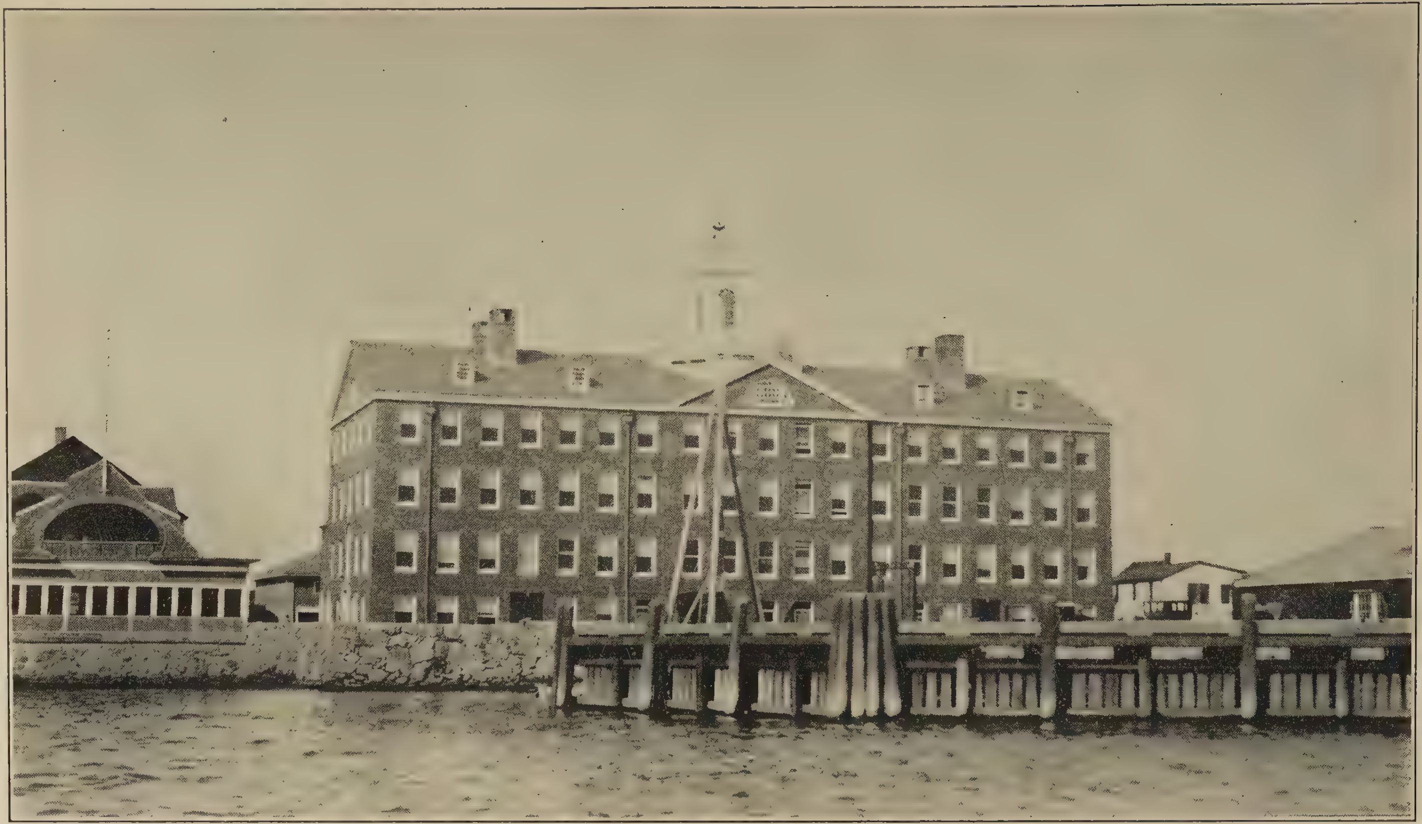
From the geologic standpoint, the *Atlantis* has only done a small amount of work during the past year. An investigation of the problem of the formation of the continental shelf off the Atlantic coast States has been in progress in the laboratory at Woods Hole. Most of the bottom samples have been collected by the *Asterias*, a 40-foot power boat also operated by the institution. But during April, 1932, two lines of stations were run by the *Atlantis* off the New Jersey coast with samples taken every 2 miles from the beach to the 1,000-fathom curve. By studying the distribution of the various sized particles composing the mud, it is hoped that something will be learned about the depth of wave action and the geologic history of the formation of the continental shelf.

The physical program of the *Atlantis* has not been so extensive that some valuable biological investigations could not be carried out at the same time. During much of the year it has been the custom to make a tow for eel larvae every second evening when the vessel was cruising in suitable regions. These tows were made to collect eel larvae for the Carlsberg Laboratory in Copenhagen. Through a study of the distribution and sizes of the young eels, their migration route from the spawning region south and east of Bermuda has been mapped out, and since their long journey back to Europe is much dependent on the currents, the eel tows are valuable to physical oceanographers because the eels make excellent drift-bottles. At the same time, a general collection of oceanic plankton has been carried out for the laboratory at Bermuda, which will eventually show more clearly the distribution of the various small floating animals found near the surface in the central North Atlantic.

The *Atlantis's* heavy winch with its 5 miles of cable was intended mainly for handling large deep-sea nets and dredges. Her cruises to date have not included any program of bottom dredging, but enough deep-sea tows have been made to test the machinery thoroughly. These tows have advanced the technique of handling large nets, besides being instructive in the problem of bringing up deep-sea fish in good condition. There is even reason to expect that in regions where the surface waters are not too warm, deep-sea animals can be brought to the surface alive, if proper precautions are taken. The cod end of the nets must be lined with some soft material so that the fish will not have their skin chafed off by the rush of water through coarse netting and the hauling speeds must be so regulated that the animals have time to " decompress " on the way to the surface. The difficulty is that fish living normally in waters having a temperature of 4° C. will be killed by being brought through the surface layers which are usually very much warmer. It would seem that in winter, if tows were made in the northern seas, this difficulty might be eliminated and the fish brought back alive.

The question of the penetration of light below the sea surface and its effect on the animal life in the upper layers of the ocean has recently received considerable impetus through the development of a reliable photometric apparatus at the Plymouth Laboratory. Similar apparatus was made for use on the *Atlantis* and during the summer of 1931 this was tried out on her crossing from Plymouth to Boston. At 13 stations careful measurements were made of the amount of light at all depths down to the limit imposed by the sensitivity of the photoelectric cells used. At the same time, through the use of a series of five closing nets, a picture of the vertical migration of the plankton was obtained which could be nicely correlated with the amount of light reaching the organisms throughout the day. Thus the phototropic effect can be studied at sea in much the same manner as in a physiological laboratory.

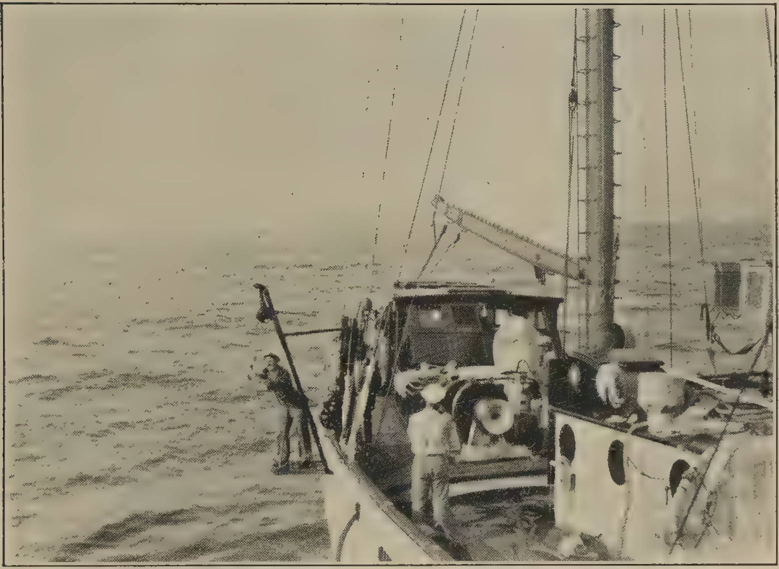
Such is a summary of the various investigations carried out from the *Atlantis* since her launching in June, 1931. This by no means represents all of the work of the Woods Hole Oceanographic Institution. A large number of marine problems have been attacked at the laboratory, and many observations have been collected by the *Asterias*, but it has been thought best to limit this account to the work done in deep water because so little is known of the problems of deep-sea oceanography.



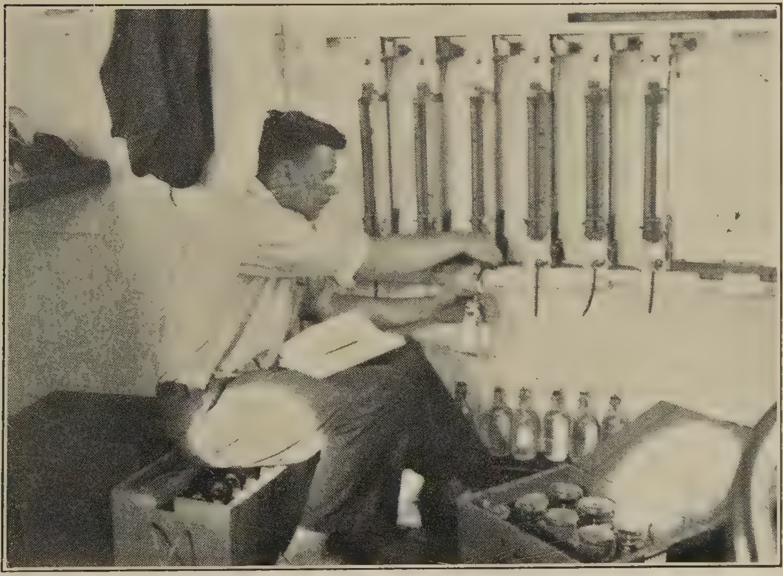
Laboratory at Woods Hole

THE "ATLANTIS"

A scientific research ship operated by the Woods Hole Oceanographic Institution.



1. WATER BOTTLES BEING ATTACHED TO THE WIRE



2. WATER SAMPLES BEING DRAWN FROM THE WATER BOTTLES IN THE DECK LABORATORY ABOARD THE "ATLANTIS"

1. PLANKTON NET BEING HOISTED ABOARD AND WASHED DOWN BY A HOSE

2. SHOWING CURRENT METER FASTENED IN THE MOUTH OF A NET TO MEASURE THE VOLUME OF WATER PASSING THROUGH IT DURING A TOW

Contribution No. 15

(Reprinted from Transactions of the American Geophysical Union,
Fourteenth Annual Meeting, 1933)

MONTHLY SEQUENCE OF SEA-SURFACE TEMPERATURE ON THE NEW YORK-SAN JUAN STEAMSHIP ROUTE

E. Monroe Harwood and Charles F. Brooks

Since April, 1926, the steamship Coamo of the Puerto Rico Line, New York to San Juan, has carried for the United States Weather Bureau a Negretti and Zambra sea-water thermograph, recording surface-temperatures at the depth of the ship's sea-water intake. With but few interruptions it has produced a continuous record of surface-temperature for each of its weekly passages, providing a fairly complete picture of the march of temperature in the ocean-surface along its route.

The Coamo thermograms have been studied and charted at the Woods Hole Oceanographic Institution and at Blue Hill Meteorological Observatory of Harvard University under the direction of Charles F. Brooks. By means of the charted temperatures of this and other steamship lines, the area from New York to San Juan has been divided into six regions of different characteristics and varying size (Fig. 1). These are the same as the regions discussed by Church a year ago (Trans. Amer. Geophys. Union, 13th annual meeting, 244-249, 1932) except that (1) his very narrow "Upwelling-Zone" just north of the "Cold Wall" and his adjoining "Cool-Water" region have been combined into a "Slope-Water" division, (2) his "Warm Area" has been renamed "Central-Atlantic Water," and (3), beyond the limit of his study, another zone, "Antilles Water," has been added.

The Coastal Water extends from the land to the edge of the continental shelf at the 100-fathom line, about 125 miles on the New York- San Juan Line. The Inner Zone, taken as 30 miles wide, has an annual temperature of 12°.5 C, ranging from an average of 3°.1 in February to 22°.8 in August--a difference of 19°.7. A Middle Zone, 65 miles wide, is but slightly warmer, 12°.7 for the year; February's average is 3°.9 and that of August, 22°.6--an annual range of 18°.7. The Outer Zone, 30 miles wide, has an annual mean of 13°.4, rising from a minimum monthly mean of 5°.3 in February to a maximum of 23°.1 in August--a range of 17°.8. As might be expected, the range of the innermost coastal-water is greatest. Examination of those instances when a marked change of temperature occurred from one week to the next indicates that changes in the surface-temperature of the general coastal-belt are first noted in the innermost strip, and appear successively in the middle and outermost parts of the belt. These changes and their progress come frequently, but are not evident in the monthly tabulation. Coastal-Water temperatures vary noticeably from the average. In general, during the five-year period 1927-1932, Coastal-Water departures have agreed with air-temperature departures of corresponding months at Blue Hill Observatory, Hyde Park, Massachusetts (see Fig. 2).

From the Coastal Water, the Coamo passes into the Slope-Water, 120 miles wide at this crossing, extending from the edge of the continental shelf to the Cold Wall, north of the Gulf Stream--the name has been used by C. O. Iselin to denote deeper waters in the same general region. The mean annual temperature is 16°6 and the mean annual range

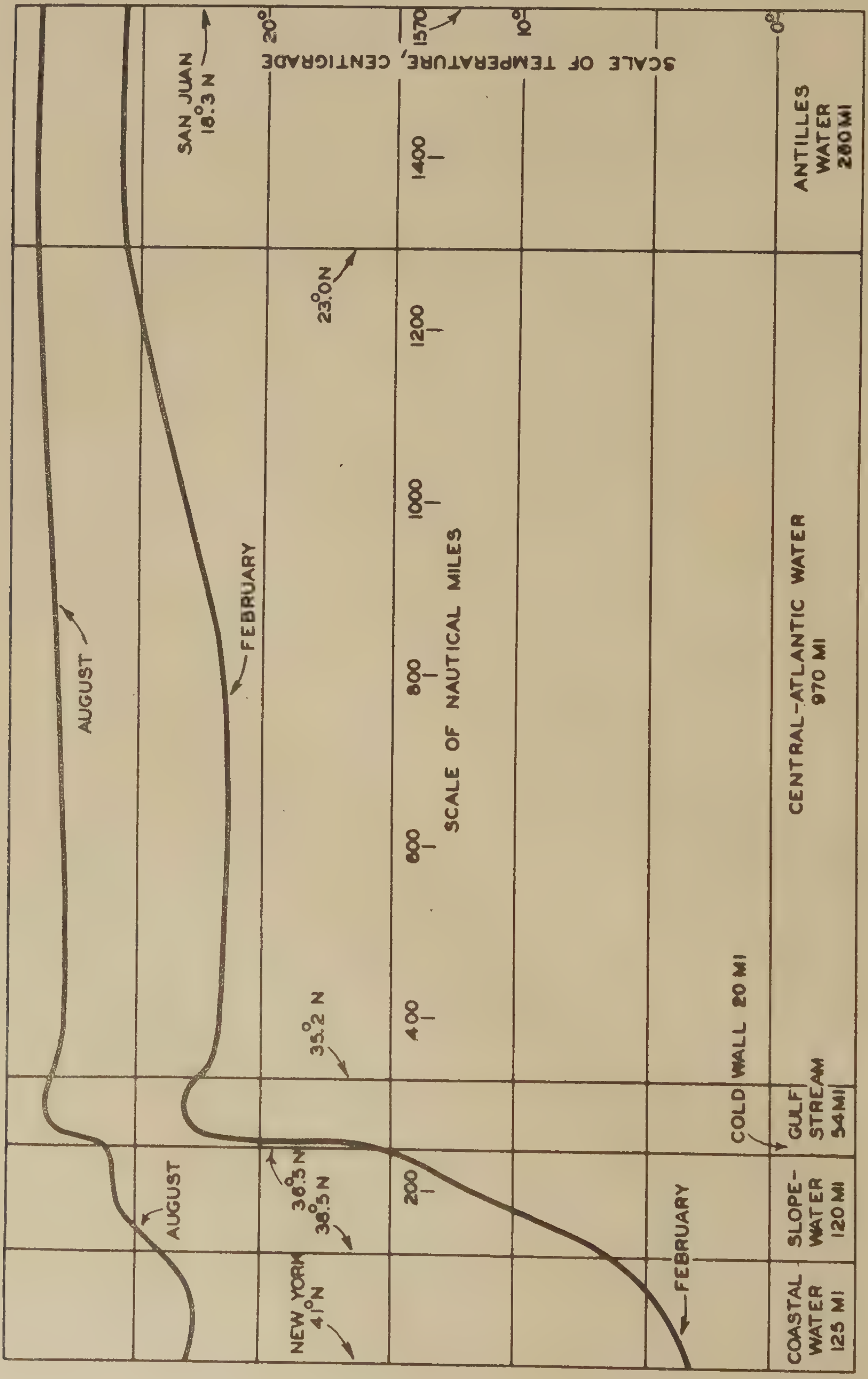


Fig. 1--Average sea-water temperature, Puerto Rico Line, 1927-32

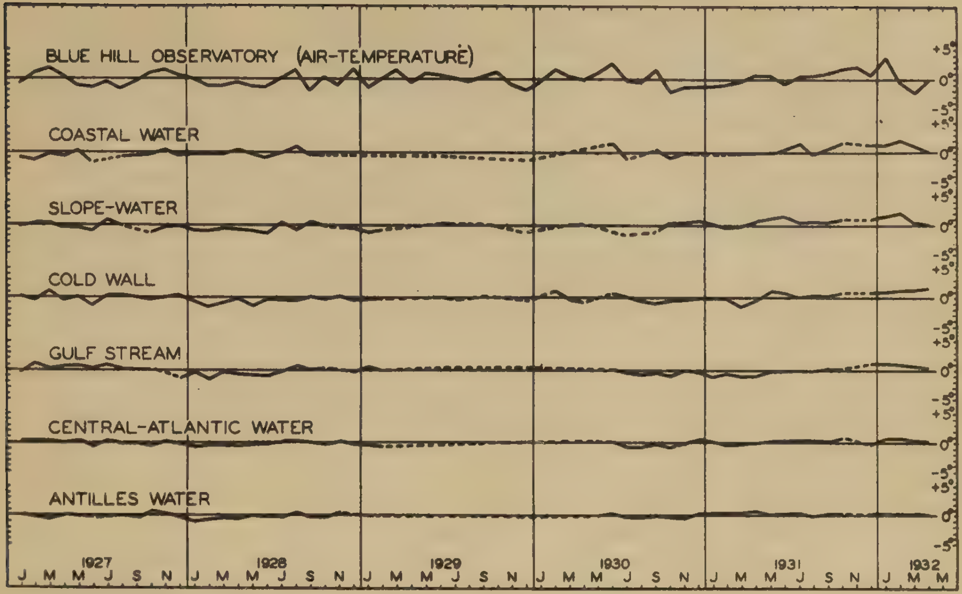


Fig. 2--Monthly surface-temperature departures in degrees Centigrade, Puerto Rico Line, New York to San Juan, 1927-32

15°.4, from 10°.2 in February to 25°.6 in August. Its departures are nearly as large as those of the Coastal Water, but do not show the same correspondence to the Blue Hill air-temperature departures. The width is quite constant, varying not more than 40 miles, usually much less.

Immediately north of the Gulf Stream, the thermograph-trace takes a sudden rise within a short space of time and within an average distance of 20 miles. The rise is 10° or more in winter but only about 3° in summer. This region has been called the Cold Wall. It partakes of the characteristics of both of its neighbors, and in its narrow width affords a change from the cool Slope-Water on the north to the constantly warm Gulf Stream on the south. The mean annual temperature is 21°.7, with the maximum, 26°.6, in August and the minimum, 17°.5, in April--an annual range of 9°.1. Departures of the monthly mean temperature of the Cold Wall are smaller than in either of the two preceding regions. In April 1931, however, it was as great as 1°.5. The width is fairly constant, seldom varying more than 10 miles from its average width of 20 miles, but occasionally changing more, with the occurrence of fingering of warm and cold water, or incursions of Gulf Stream water into the Slope-Water. Generally, however, it follows the same course, as a narrow belt, immediately north of the Gulf Stream.

At the top of the sudden rise of temperature in the Cold Wall the thermograph-trace flattens out in the Gulf Stream, at this point 53.7 miles wide on the average. The mean annual temperature is 25°.2, the annual range is 5°.8, the maximum monthly mean is 28°.3 in August, and the minimum is 22°.5 in February. While the average width during three years (1929-32) varied from 43.3 miles in September to 65.2 miles in April, the short record shows no seasonal change in width. The variations seem to be erratic. During 1929, 1930, and 1931, the width of the Gulf Stream tended to increase slightly, but the first four months of 1932 showed a slight decrease, these changes covering less than six miles. Flanked by belts of different temperature, the Gulf Stream is subject to considerable influence in both its warmth and its width, and, were it not for its constant motion, it would probably be more noticeably affected.

The Gulf Stream departures plotted against those of air-temperature at Blue Hill Observatory seem to show, in the five years, a positive relation. Breaks in the sea-temperature record make comparison for the whole period impossible, but comparison of peaks in the curve for Blue Hill with peaks in that for the Gulf Stream seems to show that these come generally in the same month, and, in two cases, in succeeding months. Comparison of weekly departures might show the relation more accurately, but the monthly figures are sufficient to suggest that a relation exists between these widely separated regions.

Table 1--Monthly air-temperatures at the Blue Hill Observatory and monthly sea-surface temperatures in degrees Centigrade on steamships of Puerto Rico Line from New York to San Juan, 1927-1932

Year	Month											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
	Blue Hill Observatory air-temperatures											
1927	-2.8	-1.3	3.7	7.7	11.9	16.8	20.9	18.5	16.8	12.8	7.5	0.2
1928	-2.0	-3.3	0.7	6.5	11.8	16.9	21.4	22.1	15.2	12.1	4.9	1.4
1929	-3.8	-2.4	3.3	6.9	14.2	18.9	21.6	19.8	17.7	10.2	4.8	-2.2
1930	-2.6	-0.4	2.1	7.4	14.2	20.8	21.4	19.8	19.2	9.3	4.5	-1.7
1931	-3.3	-3.3	1.3	7.9	13.8	17.6	22.3	20.9	18.4	13.4	8.1	0.4
1932	1.9	-2.8	-0.3	6.9	....	....	....	....	....	....	....	....
Mean	-2.1	-2.3	1.8	7.2	13.2	18.2	21.5	20.2	17.4	11.6	5.9	-0.4
	Inner Coastal Water surface-temperatures											
1927	3.6	3.1	3.9	6.1	11.7	15.6	....	22.2	20.6	18.3	12.8	7.8
1928	3.3	2.2	3.3	5.3	10.6	15.6	21.1	23.9	20.6	17.8	10.0	6.9
1929	3.3	1.7	1.7	....	....	19.4	21.9	22.2	23.3	....	8.6	5.6
1930	5.0	3.9	6.1	8.3	....	21.1	21.1	22.2	22.2	16.7	11.1	6.1
1931	3.3	2.8	5.0	8.3	11.1	18.9	23.6	23.3	22.2	20.0	13.1	8.9
1932	7.2	5.0	5.0	6.7	....	....	....	....	....	....	....	....
Mean	4.3	3.1	4.2	6.9	11.1	18.1	21.9	22.8	21.8	18.2	11.1	7.1
	Middle Coastal Water surface-temperatures											
1927	4.3	3.7	4.7	6.9	11.8	16.1	21.7	21.6	20.0	17.9	12.1	7.1
1928	4.3	3.7	4.1	6.4	11.3	16.9	21.5	23.7	20.4	16.8	10.0	8.1
1929	3.6	1.9	3.1	....	....	19.1	21.7	21.7	23.3	....	9.2	6.4
1930	4.4	5.0	6.1	8.3	....	20.0	21.2	22.9	22.2	17.1	10.8	6.5
1931	3.7	3.1	4.7	7.2	11.1	18.9	24.1	23.2	22.6	19.1	12.8	9.6
1932	7.3	5.9	5.7	7.1	....	....	....	....	....	....	....	....
Mean	4.6	3.9	4.7	7.2	11.4	18.2	22.0	22.6	21.7	17.7	11.0	7.2
	Outer Coastal Water surface-temperatures											
1927	3.9	3.3	5.0	6.7	11.1	15.6	22.8	22.8	20.6	18.1	13.3	10.3
1928	6.7	5.8	5.6	5.6	11.1	17.8	21.4	23.3	18.9	17.8	11.1	10.3
1929	7.2	3.9	4.7	....	....	20.3	21.7	22.2	22.2	....	11.1	8.9
1930	6.7	6.1	6.1	8.3	....	20.0	22.2	23.3	22.2	18.3	12.8	9.4
1931	6.1	5.6	5.3	7.8	11.1	17.2	23.3	23.9	22.2	19.4	13.9	11.1
1932	9.2	7.2	6.7	6.9	....	....	....	....	....	....	....	....
Mean	6.6	5.3	5.6	7.1	11.1	18.2	22.3	23.1	21.2	18.4	12.4	10.0
	Slope-Water surface-temperatures											
1927	11.3	10.6	10.6	10.3	12.6	17.9	23.4	25.3	....	20.2	15.9	13.9
1928	10.6	9.1	9.8	9.8	11.8	17.3	22.8	24.8	24.9	21.1	....	13.5
1929	10.1	9.4	....	....	....	19.0	22.6	....	24.6	....	....	12.9
1930	....	9.9	....	10.8	....	....	20.8	....	23.4	21.9	16.7	14.8
1931	11.6	9.9	10.3	11.4	14.3	20.9	23.2	26.5	25.0	22.6	....	15.1
1932	13.1	12.4	10.9	11.1	....	....	....	....	....	....	....	....
Mean	11.4	10.2	10.4	10.7	12.9	18.8	22.6	25.6	24.5	21.4	16.3	14.1
	Cold Wall surface-temperature											
1927	18.4	17.9	18.7	17.3	19.8	22.2	26.6	27.2	26.7	24.9	21.9	20.4
1928	17.8	16.4	17.1	17.2	18.4	22.8	25.7	26.1	26.8	24.9	21.8	19.6
1929	17.9	17.7	....	....	....	23.4	25.7	....	26.8	....	....	19.5
1930	....	19.2	17.3	16.9	....	24.2	26.2	26.0	25.6	24.4	21.3	19.8
1931	18.2	17.8	16.2	17.0	21.2	24.3	26.2	27.1	26.7	25.7	....	20.9
1932	19.2	19.2	19.0	19.1	....	....	....	....	....	....	....	....
Mean	18.3	18.0	17.7	17.5	19.8	23.4	26.1	26.6	26.5	25.0	21.7	20.1

Table 1--Continued

Year	Month											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
	Gulf Stream surface-temperatures											
1927	22.7	23.6	23.4	24.1	25.3	27.1	28.7	28.6	28.4	26.9	....	22.8
1928	22.4	21.1	22.8	22.8	23.8	25.8	27.5	29.0	28.2	26.9	....	24.1
1929	23.3	22.5	....	....	....	27.1	....	....	....	....	....	24.6
1930	....	....	....	23.7	....	27.1	27.3	27.5	27.6	25.6	24.4	23.4
1931	21.8	21.8	22.0	22.4	24.3	26.4	27.8	28.2	28.2	26.9	....	25.3
1932	24.1	23.6	23.7	23.8	....	....	....	....	....	....	....	....
Mean	22.8	22.5	23.0	23.4	24.4	26.7	27.8	28.3	28.1	26.6	24.4	24.1
	Central-Atlantic Water surface-temperatures											
1927	22.5	21.9	21.8	22.0	23.5	25.4	27.4	27.9	28.3	26.2	24.4	23.6
1928	21.4	21.1	21.1	21.1	22.7	25.4	27.1	28.3	28.3	26.4	24.6	23.4
1929	21.9	20.9	....	....	....	....	....	....	....	....	....	23.6
1930	....	....	....	22.1	....	25.8	26.2	27.1	27.7	25.8	24.3	23.6
1931	22.4	21.1	21.1	21.7	23.2	25.6	27.1	28.3	28.4	27.4	....	24.6
1932	23.2	22.4	21.9	21.9	....	....	....	....	....	....	....	....
Mean	22.3	21.5	21.4	21.8	23.1	25.6	26.9	27.9	28.2	26.3	24.4	23.8
	Antilles Water surface-temperatures											
1927	24.4	25.4	25.4	26.1	27.1	27.3	....	28.9	28.8	29.5	28.4	26.7
1928	25.1	25.1	25.3	25.4	26.8	27.5	28.2	29.3	28.8	28.6	28.2	....
1929	....	25.7	....	....	....	27.4	....	....	....	....	....	26.9
1930	....	....	....	25.7	....	27.9	28.1	28.5	28.8	28.4	27.6	27.3
1931	26.4	26.0	26.1	26.7	27.2	27.8	28.7	28.9	29.1	29.0	....	27.2
1932	26.3	25.8	26.0	26.1	....	....	....	....	....	....	....	....
Mean	25.6	25.6	25.7	26.0	27.0	27.6	28.3	28.9	28.9	28.9	28.1	27.0

Table 2--Monthly departures in degrees Centigrade from 5-year means of air-temperatures at the Blue Hill Observatory and of sea-surface temperatures on steamships of Puerto Rico Line from New York to San Juan, 1927-1932

Year	Month											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
	Blue Hill Observatory air-temperature departures											
1927	-0.7	+1.0	+1.9	+0.5	-1.2	-1.4	-0.6	-1.7	-0.6	+1.2	+1.6	+0.6
1928	+0.1	-1.0	-1.1	-0.7	-1.3	-1.3	-0.1	+1.8	-2.3	+0.5	-1.1	+1.8
1929	-1.7	-0.2	+1.5	-0.3	+1.0	+0.7	+0.1	-0.4	+0.3	-1.3	-1.1	-1.8
1930	-0.5	+1.9	+0.3	+0.2	+1.0	+2.6	-0.1	-0.4	+1.7	-2.3	-1.4	-1.3
1931	-1.2	-1.0	-0.5	+0.7	+0.7	-0.6	+0.8	+0.7	+0.9	+1.8	+2.1	+0.8
1932	+4.1	-0.6	-2.1	-0.3	....	....	....	....	....	....	....	....
	Inner Coastal Water surface-temperature departures											
1927	-0.7	-0.1	-0.3	-0.8	+0.6	-2.6	0.0	-0.6	-1.2	+0.1	+1.7	+0.7
1928	-1.0	-0.9	-0.8	-1.7	-0.6	-2.6	-0.8	+1.1	-1.2	-0.4	-1.1	-0.2
1929	-1.0	-1.4	-2.5	....	....	+1.3	0.0	-0.6	+1.6	....	-2.9	-1.5
1930	+0.7	+0.8	+1.9	+1.4	....	+3.0	-0.8	-0.6	+0.4	-1.5	0.0	-1.0
1931	-1.0	-0.3	+0.8	+1.4	0.0	+0.8	+1.7	+0.4	+0.4	+1.8	+2.0	+1.8
1932	+2.9	+1.9	+0.8	-0.3	....	....	....	....	....	....	....	....

Note: The mean yearly air-temperature departures at Blue Hill Observatory are: 1927, +0°.1; 1928, -0°.3; 1929, -0°.3; 1930, +0°.2; and 1931, +0°.4.

Table 2--Continued

Year	Month											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
Middle Coastal Water surface-temperature departures												
1927	-0.3	-0.2	0.0	-0.3	+0.4	-2.1	-0.3	-1.0	-1.7	+0.2	+1.1	-0.1
1928	-0.5	-0.2	-0.6	-0.8	-0.1	-1.3	-0.5	+1.1	-1.3	-0.9	-1.0	+0.9
1929	-1.0	-2.0	-1.6	....	....	+0.9	-0.3	-0.9	+1.6	....	-1.8	-0.8
1930	-0.2	+1.1	+1.4	+1.1	....	+1.8	-0.8	+0.3	+0.5	-0.6	-0.2	-0.7
1931	-0.9	-0.8	0.0	0.0	-0.3	+0.7	+2.1	+0.6	-0.9	+1.4	+1.8	+2.4
1932	+2.7	+2.0	+1.0	-0.1	....	....	....	....	....	....	....	....
Outer Coastal Water surface-temperature departures												
1927	-2.7	-2.0	-0.6	-0.4	0.0	-2.6	+0.5	-0.3	-0.6	-0.3	+0.9	+0.3
1928	+0.1	+0.5	0.0	-1.5	0.0	-0.4	-0.9	+0.2	-2.3	-0.6	-1.3	+0.3
1929	+0.6	-1.4	-0.9	....	....	+2.1	-0.6	-0.9	+1.0	....	-1.3	-1.1
1930	+0.1	+0.8	+0.5	+1.2	....	+1.8	-0.1	+0.2	+1.0	-0.1	+0.4	-0.6
1931	-0.5	+0.3	-0.3	+0.7	0.0	-1.0	+1.0	+0.8	+1.0	+1.0	+1.5	+1.1
1932	+2.6	+1.9	+1.1	-0.2	....	....	....	....	....	....	....	....
Slope-Water surface-temperature departures												
1927	-0.1	+0.3	+0.2	-0.4	-0.3	-0.9	+0.9	-0.2	....	-1.3	-0.4	-0.1
1928	-0.8	-1.1	-0.6	-0.9	-1.1	-1.5	+0.3	-0.7	+0.4	-0.4	....	-0.6
1929	-1.3	-0.8	....	....	....	+0.2	+0.1	....	+0.1	....	....	-1.2
1930	....	-0.3	....	+0.2	....	....	-1.7	....	-1.1	+0.5	+0.4	+0.7
1931	+0.2	-0.3	-0.1	+0.8	+1.4	+2.1	+0.6	+0.9	+0.5	+1.1	....	+1.1
1932	+1.7	+2.2	+0.5	+0.4	....	....	....	....	....	....	....	....
Cold Wall surface-temperature departures												
1927	+0.1	-0.1	+1.1	-0.2	+0.1	-1.2	+0.5	+0.6	+0.2	-0.1	+0.2	+0.4
1928	-0.5	-1.6	-0.6	-0.3	-1.4	-0.3	-0.4	-0.5	+0.3	-0.1	+0.1	-0.4
1929	-0.3	-0.3	....	....	....	0.0	-0.4	....	+0.3	....	....	-0.5
1930	....	+1.2	-0.3	-0.6	....	+0.8	+0.1	-0.5	-0.9	-0.6	-0.4	-0.2
1931	-0.1	-0.2	-1.5	-0.5	+1.4	+0.8	+0.1	+0.5	+0.2	+0.7	....	+0.9
1932	+0.9	+1.2	+1.3	+1.6	....	....	....	....	....	....	....	....
Gulf Stream surface-temperature departures												
1927	-0.1	+1.1	+0.4	+0.8	+0.9	+0.4	+0.9	+0.3	+0.3	+0.3	....	-1.3
1928	-0.4	-1.4	-0.2	-0.6	-0.7	-0.9	-0.3	+0.7	+0.1	+0.3	....	-0.1
1929	+0.5	0.0	....	....	....	+0.4	....	....	....	....	....	+0.4
1930	....	....	....	+0.3	....	+0.4	-0.5	-0.8	-0.5	-1.0	-0.0	-0.2
1931	-1.1	-0.7	-1.0	-0.9	-0.2	-0.3	0.0	-0.1	+0.1	+0.3	....	+1.2
1932	+1.2	+1.1	+0.7	+0.4	....	....	....	....	....	....	....	....
Central-Atlantic Water surface-temperature departures												
1927	+0.2	+0.4	+0.4	+0.2	+0.4	-0.2	+0.4	0.0	+0.1	-0.3	0.0	-0.2
1928	-0.9	-0.4	-0.4	-0.7	-0.4	-0.2	+0.1	+0.4	+0.2	-0.1	+0.2	-0.4
1929	-0.4	-0.6	....	....	....	....	....	....	....	....	....	-0.2
1930	....	....	....	+0.3	....	+0.3	-0.8	-0.8	-0.4	-0.7	-0.1	-0.2
1931	+0.1	-0.4	-0.4	-0.1	+0.1	+0.1	+0.2	+0.4	+0.3	+0.9	....	+0.8
1932	+0.9	+0.9	+0.5	+0.2	....	....	....	....	....	....	....	....
Antilles Water surface-temperature departures												
1927	+0.1	-0.2	-0.3	+0.1	+0.1	-0.3	....	0.0	-0.1	+0.6	+0.3	-0.3
1928	-0.8	-0.6	-0.4	-0.6	-0.2	-0.1	-0.2	+0.4	-0.1	-0.3	+0.1	....
1929	....	+0.1	....	....	....	-0.2	....	....	....	....	....	-0.1
1930	....	....	....	-0.3	....	+0.3	-0.2	-0.4	-0.1	-0.5	-0.5	+0.3
1931	+0.4	+0.4	+0.4	+0.7	+0.2	+0.2	+0.4	0.0	+0.2	+0.1	....	+0.2
1932	+0.3	+0.2	+0.3	+0.1	....	....	....	....	....	....	....	....

CORRECTED TABLE TO REPLACE TABLE 3, PAGE 179, TRANSACTIONS OF THE AMERICAN GEOPHYSICAL UNION, FOURTEENTH ANNUAL MEETING

Table 3--Widths in nautical miles of six temperature-divisions for route New York to San Juan of Puerto Rico Line, 1929-1932

Year	Month												Year
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	
						Coastal Water							
1929	125	128	128	...	...	114	118	...	118	...	117	118	121
1930	123	117	117	116	...	117	118	117	116	117	122	121	118
1931	118	117	117	117	117	117	117	116	117	116	123	117	117
1932	119	117	117	...	...	...	...	...	...	...	...	...	...
Mean	121	120	120	117	117	116	118	117	117	117	121	119	118
						Slope Water							
1929	65	93	97	...	...	...	102	...	132	...	111	103	101
1930	96	105	120	111	...	72	93	105	90	108	100	78	98
1931	102	75	118	150	155	139	106	141	139	147	100	97	122
1932	117	129	146	134	...	...	...	...	...	...	...	...	...
Mean	95	100	121	132	155	106	100	123	120	128	104	93	115
						Cold Wall							
1929	18	18	37	...	...	24	22	...	8	...	14	20	20
1930	15	8	32	18	...	42	17	7	30	15	30	42	23
1931	21	7	26	14	14	20	32	22	23	13	35	40	22
1932	20	24	21	25	...	...	...	...	...	...	...	...	...
Mean	19	14	29	19	14	29	24	15	20	14	26	34	21
						Gulf Stream							
1929	55	51	47	...	...	41	40	...	43	...	31	37	43
1930	47	39	42	66	...	32	70	36	30	42	41	60	46
1931	45	34	35	52	52	59	42	80	45	63	50	37	50
1932	49	41	39	50	...	...	...	...	...	...	...	...	...
Mean	49	41	41	56	52	44	51	58	39	53	41	45	48
						Central-Atlantic Water							
1929	868	872	832	...	...	883	790	...	665	...	930	832	834
1930	967	894	840	898	...	825	757	720	898	797	840	807	840
1931	874	973	978	892	794	765	833	764	804	808	905	845	853
1932	802	867	808	795	...	...	...	...	...	...	...	...	...
Mean	878	902	832	862	794	824	793	742	789	803	892	828	828
						Antilles Water							
1929	253	220	240	...	...	268	263	...	384	...	195	267	261
1930	135	212	175	175	...	295	322	400	215	272	195	207	237
1931	216	178	109	122	240	285	226	257	257	233	222	246	216
1932	255	212	266	262	...	...	...	...	...	...	...	...	...
Mean	215	206	198	186	240	283	270	329	285	253	204	240	242

Note: All figures relating to widths in the text should be changed according to the distances given in this corrected Table.

South of the Gulf Stream, the *Coamo* steams through the western end of the broad Central-Atlantic Water, at this crossing 970 miles wide, with a mean temperature of 24°4 and a range of 6°8. Its maximum, 28°2, comes in September and its minimum, 21°4, in March. Central-Atlantic Water monthly departures are small, and seem to be larger in winter than in summer. With the fluctuating Gulf Stream on one side, and the poorly defined Antilles Water on the other, its width, like that of the Gulf Stream, varies erratically. Yearly averages of width, however, are quite constant, varying less than 25 miles from the average of 970 miles for the period from 1929 through April 1932. There is some indication of lesser widths in summer and of greater widths in winter.

The final stretch on the route to Puerto Rico, the Antilles Water, extends from the southern edge of the Central-Atlantic Water, about 280 miles to San Juan. The line separating the two divisions is poorly marked; the Antilles Water is distinguished only by a flattening of the thermograph-trace to almost a horizontal line. From this we may expect rather constant conditions, and we find the mean annual temperature to be 27°3, with the maximum, 28°9, coming in August or September and the minimum, 25°6, in February. The range is but 3°3. The monthly departures are the smallest of the six divisions, being, for the most part, almost imperceptible. They seem to be largest in winter or spring. With its northern boundary so poorly defined, the width of this area fluctuates considerably, the changes being generally complementary to those of the Central-Atlantic Water. As noted in connection with the discussion of the Gulf Stream, the record suggests a relation between departures of this area and those of air-temperature at Blue Hill. The peaks of the sea-temperature curve come in the same month as, or in the month before, the corresponding ones in the Blue Hill record. In four positive peaks of temperature during 1927-1932, and five negative peaks, the three places, Antilles Water, Gulf Stream, and Blue Hill, show essential concurrence in the character of departures.

These monthly temperatures have value, in that they provide in fairly concise form the essential data, and although they do not permit consideration of the details, they suggest major changes. The western Atlantic, which washes our eastern shores, influences our climate here on the east coast. Knowledge of the ocean-temperatures, both at the surface and at greater depths may shed light on the seasonal variations of weather in temperate eastern North America.

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Contribution No. 16

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THE OXYGEN DISSOCIATION CURVES OF SOME BLOODS CONTAINING HEMOCYANIN

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TWENTY FIGURES

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"To say that every worker on haemoglobin has had a pet theory of the way in which that substance unites with oxygen would of course be a gross exaggeration, nevertheless the lure of this subject has been remarkable—perhaps 'remarkable' is the wrong word because the importance of the subject is so great." (Barcroft, '28.)

The most immediate interest in the study of hemocyanin arises from the fact that this substance, together with hemoglobin, chlorocruorin, and hemerythrin, form a class of compounds unique in uniting with oxygen in a way so labile that they serve in the transport of oxygen by the blood. For the study of this property the hemocyanins possess the advantage of forming a numerous group of related compounds differing among themselves in the quantitative aspects of their properties. The development of a precise spectrophotometric method of estimating the quantity of oxyhemocyanin present in a solution has made it possible to measure the oxygen dissociation curves of the blood of a number of invertebrates with a precision not heretofore obtained. The results of these studies are set forth in the present paper together with a theoretical treatment which enables one to define the conditions of equilibrium under certain stated conditions with the aid of a small number of numerical constants and which adds one more to the number of theories of the way in which such substances unite with oxygen.

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[1] Contribution no. 16.

It has been shown by Stedman and Stedman ('28) that the equilibrium of oxygen with the dialyzed hemocyanin of Helix pomatia is described by the mass law on the assumption that one molecule of hemocyanin reacts with one molecule of oxygen. A similar relation has been demonstrated for the purified hemocyanin of Limulus polyphemus (Redfield, '30) and Busycon canaliculatum (Redfield and Ingalls, '32). Similar relations are described by Stedman and Stedman ('26 a) for the dialyzed blood of the lobster Homarus vulgaris at pH 9.65, and it will be shown in the present paper that the blood of Busycon displays this property when acidified to a pH of 5.79. These observations appear to justify the attempt to account for the form of the oxygen dissociation curve of solutions containing hemocyanin by means of some modification of the mass law. All other oxygen dissociation curves which have been accurately described depart from the simple hyperbolic form required by the mass law if it is supposed that the oxygen-binding groups of the hemocyanin are situated upon separate molecules or are so separated in their position in a common molecule that their reactions are independent of one another. The departure of these curves from the hyperbolic form may be taken as an indication of some sort of interdependence in the behavior of associated prosthetic groups. In the theories of the union of hemoglobin with oxygen this interdependence has been explained by the assumption that the hemoglobin molecule consisted of aggregates of several oxygen-binding units so constituted that the oxygenated form was only stable when all of the associated groups were combined with oxygen, as in the theory of Hill ('10) and its modifications by others, or that the oxygenation of the four prosthetic groups of the hemoglobin molecule takes place as a series of steps, each characterized by a distinctive oxygen dissociation constant (Adair, '25 a and b).

In the case of the former theory, the shape of the oxygen dissociation curve is described by the equation—

$$y = \frac{Kx^n}{1+Kx^n}$$

where y is the fraction of the respiratory protein in the oxygenated condition, K is the oxygen equilibrium constant, x is the partial pressure of oxygen, conventionally measured in millimeters of mercury, and n is the number of prosthetic groups so associated as to form a stable oxygenation product. Theoretically, n should be an integer, although it has commonly been assumed, in order to account for the findings with hemoglobin, that n is the average value of the number of units in each aggregate (Barcroft, '28).[2] Under certain circumstances, the oxygen dissociation curve of the lobster Homarus americanus and of the squid Loligo pealei is satisfactorily described by Hill's equation on the assumption that n is equal to 2 and, under other conditions, that n is equal to 4. This is illustrated by data presented in figure 1 (curves A, B, F, G, H, I) in which the logarithm of the ratio of oxyhemocyanin to reduced hemocyanin is plotted against the logarithm of the oxygen pressure. According to this method of presentation, data which conform to Hill's equation should appear as a straight line with a slope equal to the value of n. These selected cases demonstrate that with the aid of Hill's equation the mass law may be extended to account for some of the more complicated situations existing in the blood.

A striking fact brought out by the study of the oxygen dissociation curves of hemocyanin is that various factors which influence the equilibrium between hemocyanin and oxygen do so not only by altering the general position of the oxygen dissociation curve with regard to the scale of oxygen pressures, but also by changing the 'shape' of the curve describing the equilibria. This is shown especially clearly by the observations of Stedman and Stedman ('26, a and b) upon crustacean hemocyanin as well as by the numerous measurements presented in this paper. The nature of the phenomena respon-

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[2] It may be pointed out, however, that if the solution consists of a mixture of aggregates of different size, i.e., each characterized by a different value of n, the resulting oxygen dissociation curve cannot be described by giving n a constant non-integral value. The equation developed in the present paper is derived by writing a term for each possible state of the hemocyanin characterized by an integral value of n. Such a series of terms cannot be reduced to the form of Hill's equation.

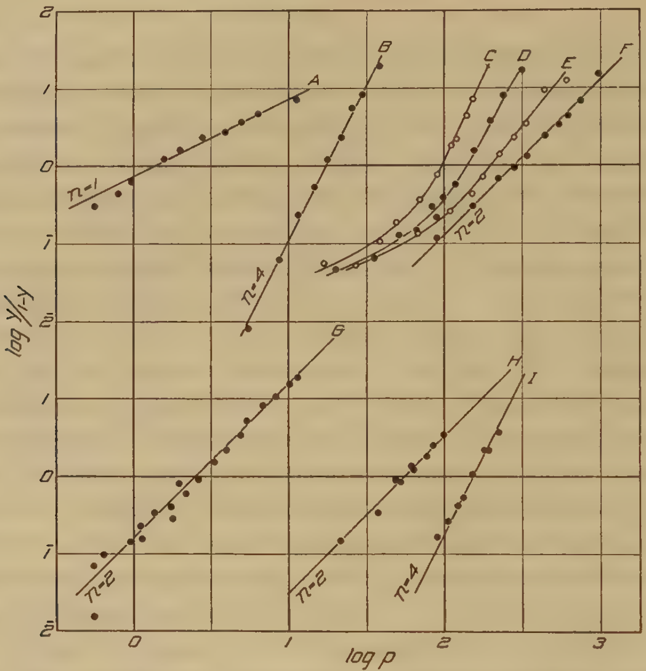

Fig. 1 Oxygen dissociation curves of some bloods containing hemocyanin plotted in accordance with the logarithmic form of Hill's equation. Ordinate, logarithm of the ratio of oxyhemocyanin to reduced hemocyanin; abscissa, logarithm of oxygen pressure, (measured in millimeters of mercury). A. Serum of Busycon canaliculatum, pH 5.79, temperature, 25°C. B. Serum of Homarus americanus diluted with phosphate buffer, pH 8.08, temperature, 25°C. C. Serum of Homarus americanus diluted with phosphate buffer, pH 7.27, temperature, 25°C. D. Serum of Homarus americanus diluted with phosphate buffer, pH 7.11, temperature, 25°C. E. Serum of Homarus americanus diluted with phosphate buffer, pH 6.82, temperature, 25°C. F. Serum of Homarus americanus diluted with phosphate buffer, pH 6.42, temperature, 25°C. G. Serum of Loligo pealei diluted with sea water, pH 9.03, temperature, 15°C. H. Serum of Loligo pealei undiluted, pCO_2, 7.35 mm., temperature, 5°C. I. Serum of Loligo pealei undiluted, pCO_2, 14.7 mm., temperature, 25°C.

sible for the change in the shape of the oxygen dissociation curve of hemocyanin is to be sought by examining the intermediate conditions through which the curves pass as one goes from a state in which n has one integral value to that in which n has another integral value. In the case of the blood of the lobster, the curves obtained at reactions intermediate between those at which n is equal to 2 and 4, respectively, are of a sigmoid character, as shown in figures 7, 8, and 9. The data obtained at such intermediate reactions are plotted according to the logarithmic scheme in figure 1 (curves C, D, E). It is clear that the line drawn through the experimental points is not straight and that consequently n is not constant. The transition does not occur as one might expect if, in accordance with the Hill equation, the value of n changed with pH, but remained constant at any given pH value. Aside from this the curves obtained from lobster serum do not have a sufficiently definite character to indicate what is taking place. On the other hand, the examination of the blood of other species yields curves which suggest the sort of phenomenon which must be taken into account in explaining the change in the shape of the oxygen dissociation curve with such factors as hydrogen ion concentration. In figures 2 and 3 are presented data on the oxygen dissociation curve of the serum of Limulus polyphemus at pH 7.53 and Busycon canaliculatum at pH 8.35. These curves possess a definitely undulatory character, suggesting that the bloods consist of mixtures of two or more components, each of which reacts with oxygen independently of the others and reaches an equilibrium described by a distinct oxygen dissociation curve.

In order to develop this view it may be assumed that each component reacts with oxygen in accordance with Hill's equation, but that the components are each characterized by a different integral value of n. We may designate the oxygen equilibrium constants of the forms characterized by values of n equals 1, 2, 3, 4, etc., by K_1, K_2, K_3, K_4, etc., and the fraction of the total oxygen bound by each of these forms as α_1, α_2, α_3, α_4, etc. The fraction of the total hemocyanin present in the oxygenated condition, y, is consequently given by the equation—

$$y = \frac{a_1K_1x^1}{1+K_1x^1} + \frac{a_2K_2x^2}{1+K_2x^2} + \frac{a_3K_3x^3}{1+K_3x^3} + \frac{a_4K_4x^4}{1+K_4x^4} \qquad (1)$$

in which x is the partial pressure of oxygen. By means of this expression, it has been possible to describe all of the oxygen dissociation curves which we have succeeded in measuring. With the exception of certain curves for Busycon serum at alkaline reactions and one curve for Limulus serum at low temperature, it has not been necessary to assume the presence of more than two components, so that the curves can be described by the simpler equations—

$$y = \frac{a_1K_1x^1}{1+K_1x^1} + \frac{a_2K_2x^2}{1+K_2x^2} \qquad (2)$$

or—

$$y = \frac{a_2K_2x^2}{1+K_2x^2} + \frac{a_4K_4x^4}{1+K_4x^4} \qquad (3)$$

It has never proved necessary to assume the presence of a form characterized by n equals 3, although there is nothing to exclude the possibility of the existence of such a component.

In the following pages the data obtained from studies of the blood of the lobster Homarus americanus, the squid Loligo pealei, the horseshoe crab Limulus polyphemus, and the conch Busycon canaliculatum, under various conditions of hydrogen ion concentration and temperature, are presented together with the results of an analysis of the form of these curves in terms of the foregoing equations.

EXPERIMENTS

The method employed in measuring the rate of oxygenation of the solutions was the spectrophotometric technique previously described (Redfield, '30). The bloods were obtained in sufficient quantity to serve for the determination of a considerable number of dissociation curves and were preserved for periods of 2 or 3 weeks with toluene in the cold. The sera of Limulus, Loligo, and Homarus do not appear to alter their properties during this period. In the case of Busycon there was some evidence of minor changes in the shape of the dissociation curve. There is, however, no reason to suspect that the form of the curve changed during the course of a single experiment.

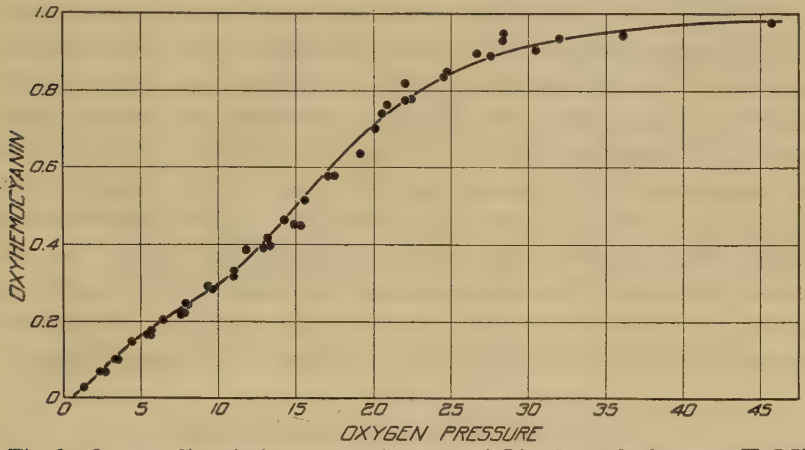

Fig. 2 Oxygen dissociation curve of serum of Limulus polyphemus, pH 7.52, temperature, 25°C. Ordinate, fraction of hemocyanin present as oxyhemocyanin; abscissa, oxygen pressure measured in millimeters of mercury. Curve calculated employing the constants: $\alpha_2 = 0.27$, $\alpha_4 = 0.73$, $K_2 = 0.06$, $K_4 = 0.00001$.

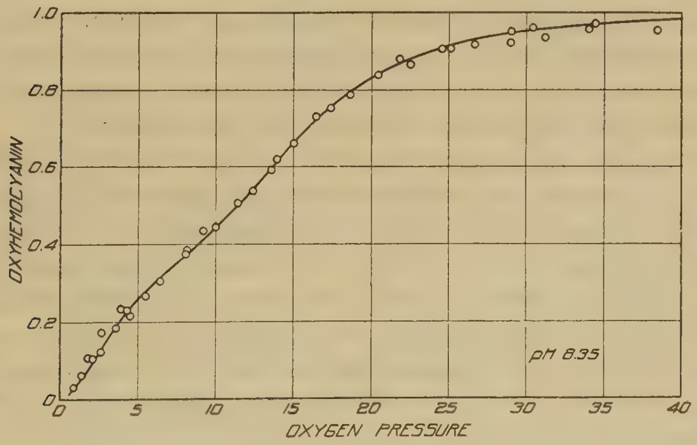

Fig. 3 Oxygen dissociation curve of serum of Busycon canaliculatum, pH 8.35, temperature, 25°C. Ordinate, fraction of hemocyanin present as oxyhemocyanin; abcsissa, oxygen pressure measured in millimeters of mercury. Curve calculated from constants shown in table 4.

The various bloods were diluted either with water, sea water, phosphate, or acetate buffers, or an artificial electrolyte solution approaching in composition the electrolytes of the blood. The hydrogen ion concentration of these solutions was adjusted by the addition of sodium hydroxide or hydrochloric acid and was measured upon samples of the reduced solution by means of the hydrogen electrode. The diluted solutions were prepared the day preceding the experimental determinations and were filtered immediately before being used. In the case of the more alkaline solutions, a precipitate of magnesium hydroxide formed when the solutions were mixed and was removed by filtration. This phenomenon limited the alkaline range of the experimental series. In the case of the squid, experiments were also made with undiluted serum, in which the hydrogen ion concentration was varied by equilibrating with different pressures of carbon dioxide.

The blood of Limulus polyphemus

The blood of several horseshoe crabs was combined, freed from the massive clot by shaking, centrifuging, and finally filtering. For each day's work 20 cc. of serum was mixed with 35 cc. sea water to which 5 cc. of distilled water containing various amounts of HCl or NaOH had been added. The dilution of the serum was consequently one-third, and the dilutant was a fluid very similar in saline composition, but of some 12 per cent lower salinity than the serum.

The oxygen dissociation curves obtained at 25°C. and at various hydrogen ion concentrations are presented in figures 4 and 5. These curves include experiments made on two different stock solutions. In general, experiments made with different bloods were in agreement when compared at similar hydrogen ion concentration and temperature. The curve shown in figure 2 is exceptional in that it is similar to the curves usually obtained at pH 8.2, although this material had a pH of 7.52.

The blood was diluted in order that various amounts of acid or alkali might be added to it without varying the concentration of hemocyanin in the solutions finally employed. The

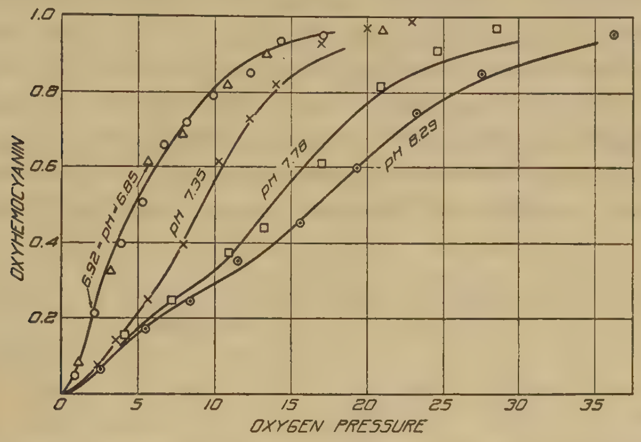

Fig. 4 Oxygen dissociation curves of serum of Limulus polyphemus, diluted with sea water, at pH values less than 8.3. Temperature, 25°C. Ordinate, fraction of hemocyanin present as oxyhemocyanin; abscissa, oxygen pressure measured in millimeters of mercury.

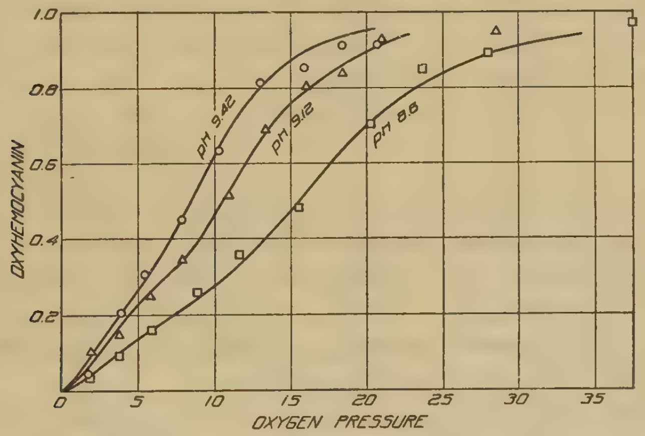

Fig. 5 Oxygen dissociation curves of serum of Limulus polyphemus, diluted with sea water, at pH values greater than 8.3. Temperature, 25°C. Ordinate, fraction of hemocyanin present as oxyhemocyanin; abscissa, oxygen pressure measured in millimeters of mercury. Curves calculated from constants shown in table 1.

degree of dilution was determined by the dimensions of the tonometer vessels employed in measuring the extinction coefficient of the blood in which the length of the T-piece was 3.3 cm. In the case of Limulus serum, by employing some especially constructed tonometers, curves were obtained without diluting the blood. Curves obtained at pH 6.86 and 7.38 agreed closely in shape and position with the curves shown in figure 4 obtained from diluted blood at similar reactions.

The data represented in figure 4 show that with decreasing alkalinity the affinity of the hemocyanin for oxygen decreases up to a pH of about 8.3, thus confirming with greater accuracy the original observations of Redfield, Coolidge, and Hurd ('26) and Hogben and Pinhey ('27) upon this material. At higher alkalinities the affinity for oxygen increased again (fig. 5). The curves show also a shape which changed definitely with hydrogen ion concentration.

TABLE 1

Constants used in calculating the oxygen dissociation curves of Limulus serum. Dilution 1:3 with sea water. Temperature, 25°C.

pH	K_2	K_4	a_2	a_4
6.05	0.15	0.15×10^{-3}	0.7	0.3
6.43	0.15	0.15×10^{-3}	0.7	0.3
6.92	0.10	0.14×10^{-3}	0.7	0.3
7.18	0.07	0.11×10^{-3}	0.5	0.5
7.35	0.06	0.08×10^{-3}	0.3	0.7
7.61	0.05	0.04×10^{-3}	0.3	0.7
7.78	0.05	0.014×10^{-3}	0.3	0.7
8.00	0.05	0.010×10^{-3}	0.3	0.7
8.29	0.05	0.006×10^{-3}	0.3	0.7
8.60	0.05	0.010×10^{-3}	0.25	0.75
9.12	0.08	0.04×10^{-3}	0.3	0.7
9.42	0.12	0.10×10^{-3}	0.3	0.7

In order to formulate these effects with greater precision, the data have been fitted as well as could be with curves, shown in figures 4 and 5, calculated from equation 3, which is the simplest reduced form of equation 1 that has proved appropriate. The constants employed are given in table 1.

The effect of temperature. The oxygen dissociation curves of Limulus have been determined at temperatures ranging from 5°C. to 44°C. For this study a stock solution was prepared, by diluting 200 cc. of fresh serum with 360 cc. sea water to which 40 cc. 0.06 normal NaOH had been added. When freshly prepared on August 18th the reaction of the solution was pH 8.33. The pH was checked during the course of the experiments and at their termination (on September 5th and again on September 14th) and was found to be unchanged. This reaction was chosen because it represents a state in which the oxygen dissociation constants are minimal and may be expected to change to a minimal degree with change in pH. An attempt was made to determine the effect of temperature upon the pH of this solution by adding a known quantity of cresol red to a sample of it, and estimating the degree of ionization of the dye from the absorption coefficient after correcting for the absorption of light by the hemocyanin. When allowance was made for the effect of temperature upon the indicator (Buch, '29), it appeared that a change in temperature from 1.5°C. to 46°C. decreased the pH not more than 0.2 pH units. We consider that the effects observed in the experiments are due to direct influences of temperature upon the oxygen equilibrium and are not secondary effects arising from thermogenic alterations in the hydrogen ion concentration.

In working at temperatures other than that of the laboratory it was found essential to keep the tonometers close to the equilibrium temperature while the spectrophotometric measurements were made. Otherwise an appreciable error was introduced through the change in distribution of oxygen between the dissolved and combined state. In order to avoid this error, the tonometers were surrounded by a copper jacket containing water of the necessary temperature while the measurements were in progress. A serious difficulty arose from the tendency for fog to form on the glass faces of the tonometers when these were colder than the air. This difficulty was overcome by dipping the end of the tonometer into chilled alcohol just before the measurements were made, so that the faces were covered with a continuous film of this liquid.

The oxygen dissociation curves obtained with Limulus serum at 5°, 15°, 25°, 35°, and 44° are shown in figure 6. With rising temperature the equilibrium is shifted, as has been shown by Hogben ('26) and Hogben and Pinhey ('26, '27) and Brown ('33) for various hemocyanin systems. The shape of the oxygen dissociation curves is altered by a change in temperature, though the definitely undulatory character of the curves persists.

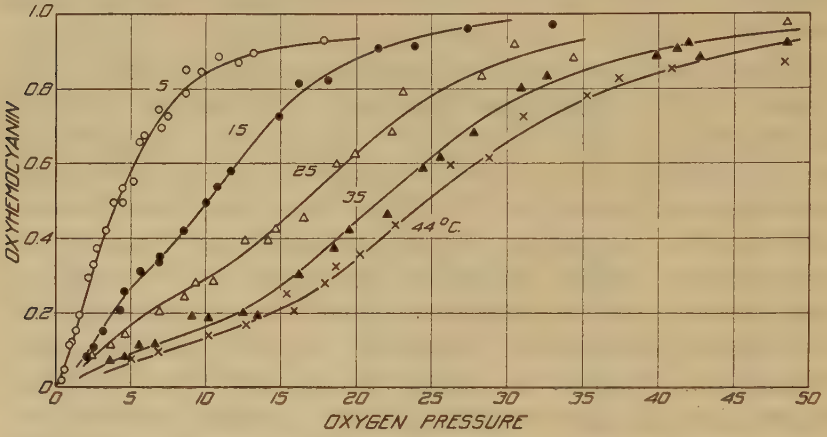

Fig. 6 Oxygen dissociation curves of serum of Limulus polyphemus, diluted with sea water at various temperatures; pH 8.33. Ordinate, fraction of hemocyanin present as oxyhemocyanin; abscissa, oxygen pressure measured in millimeters of mercury. Curves calculated from constants shown in table 2.

The curves drawn through the experimental measurements have been calculated from equations 1 and 3. The constants are given in table 2.

The blood of Homarus americanus

Two series of preparations of lobster blood have been studied. The blood was drawn from freshly caught animals, prevented from clotting by stirring, freed from the carotinoid pigment by shaking with chloroform, and filtered. The first preparation (no. 6) was diluted by adding 30 cc. of serum to a mixture of 25 cc. balanced salt solution and 5 cc. of water containing a varying quantity of HCl or NaOH. The balanced salt solution had the composition: NaCl 23.4 gm., KCl. 0.75 gram., $CaCl_2$ 2.2 gm., H_2O to 1000 cc. This solution was devised from the analyses of lobster blood by Macallum ('10) with a view to obtaining a medium similar to lobster blood, but with the magnesium replaced by calcium. Crustacean hemocyanin, as shown by Stedman and Stedman ('26, a and b), Hogben ('26), varies greatly in its affinity for oxygen at different hydrogen ion concentrations. It was found that these preparations did not give precise results at those reactions in which this variation is marked, and consequently a second series of preparations was made in which the blood was diluted with prosphate and acetate buffers. In preparation 7 the proportion of serum to buffer solution was 27:36; in preparations 8 and 10 it was 1:1; and in preparation 9 it was 2:1. Since the curves obtained from the various preparations fall together in a continuous series, the different degrees of dilution have no demonstrable effect on the oxygen equilibrium. The phosphate and acetate buffers were planned to have an ionic strength of 0.5 at each pH value, and were devised from the tables provided by Cohn ('27) and Cohn, Heyroth, and Menkin ('28).

TABLE 2

Constants used in calculating the oxygen dissociation curves of Limulus serum. pH 8.33. Dilution, 1:3 with sea water

TEMPERATURE	K_1	K_2	K_4	a_1	a_2	a_4
Degrees						
5	0.25	0.10	0.6×10^{-3}	0.3	0.5	0.2
15	...	0.07	0.03×10^{-3}	0	0.4	0.6
25	...	0.05	0.006×10^{-3}	0	0.3	0.7
35	...	0.03	0.003×10^{-3}	0	0.2	0.8
44	...	0.02	0.0018×10^{-3}	0	0.18	0.82

The data obtained from the preparations diluted with the unbuffered salt solution are not shown, but the constants which yield curves fitting the data are recorded in table 3. The data obtained with the buffered solutions at alkaline ranges (pH 8.77 to 7.27) are shown in figure 7; at nearly neutral reactions (pH 7.27 to 6.42) in figure 8, and at acid reactions in figure 9. The great change in the position of the curves which alteration in the hydrogen ion concentration brings about necessitates the presentation of the data in separate figures differing in the scale along which oxygen pressure is measured. Moreover, at acid reactions the direction in which the change occurs as a function of pH is reversed, as previously noted by Hogben ('26) and Stedman and Stedman ('26 a and b) for other crustaceans.

TABLE 3

Constants used in calculating the oxygen dissociation curves of diluted lobster serum. Temperature, 25° C.

DILUENT	PREPARATION NO.	pH	K_1	K_2	K_4	a_1	a_2	a_4
Acetate buffer	10	4.91	?	3.16×10^{-4}		?	?	0
	10	5.25	?	4.8×10^{-5}		?	?	0
Phosphate buffer	8	5.65	1.5×10^{-2}	2.0×10^{-5}	....	0.1	0.9	0
	9	5.83	1.0×10^{-3}	1.2×10^{-5}	....	0.1	0.9	0
	10	6.06	1.0×10^{-3}	1.0×10^{-5}	....	0.1	0.9	0
	8	6.42	....	1.2×10^{-5}	....	0	1.0	0
	9	6.73	....	1.8×10^{-5}	0.6×10^{-10}	0	0.85	0.15
	8	6.82	....	8.0×10^{-5}	2.0×10^{-10}	0	0.40	0.60
	9	7.10	....	4.0×10^{-4}	2.0×10^{-9}	0	0.20	0.80
	8	7.27	....	7.0×10^{-4}	1.0×10^{-8}	0	0.15	0.85
	8	7.72	....	....	4.27×10^{-6}	0	0	1.0
	7	8.08	....	....	1.0×10^{-5}	0	0	1.0
	9	8.77	....	....	1.09×10^{-3}	0	0	1.0
Unbuffered chloride solution	6	7.22	....	1.0×10^{-3}	5.0×10^{-8}	0	0.4	0.6
	10	7.54	....	....	2.5×10^{-6}	0	0	1.0
	6	7.72	....	....	1.0×10^{-5}	0	0	1.0
	10	8.17	....	....	1.0×10^{-4}	0	0	1.0

In dealing with preparations in which the affinity for oxygen is low—as is the case with lobster hemocyanin at neutral reaction—a practical difficulty arises. The hemocyanin does not appear to be fully oxygenated even in the presence of an

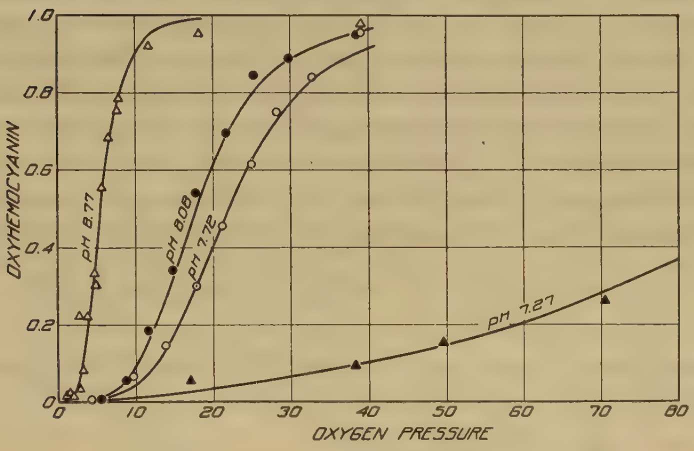

Fig. 7 Oxygen dissociation curves of serum of Homarus americanus, diluted with phosphate buffer, at alkaline reactions at which n = 4; temperature, 25°C. Ordinate, fraction of hemocyanin present as oxyhemocyanin; abscissa, oxygen pressure measured in millimeters of mercury. Curves calculated from constants shown in table 3.

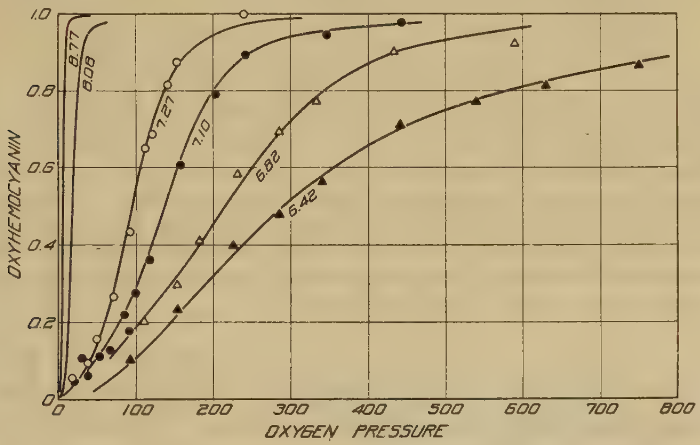

Fig. 8 Oxygen dissociation curve of serum of Homarus americanus, diluted with phosphate buffer, at reactions at which the affinity for oxygen decreases with increasing acidity. Temperature, 25°C. Ordinate, fraction of hemocyanin present as oxyhemocyanin; abscissa, oxygen pressure measured in millimeters of mercury. Curves drawn from calculations made with constants shown in table 3.

atmosphere of oxygen. This is evidenced by the fact that the difference between the absorption coefficient of oxygenated and reduced solutions diminishes in the less alkaline preparations. In calculating the percentage saturation of the various preparations, it has been assumed that the absorption of light by the completely oxygenated prosthetic groups at any pH would be equal to that observed in alkaline preparations of the same material. The magnitude of this correction is shown by the distance of the curves from the 100 per cent abscissa at oxygen pressures of about 740 mm. This assumption neglects the possibility that the absorption of light by oxyhemocyanin may actually diminish in acid media.

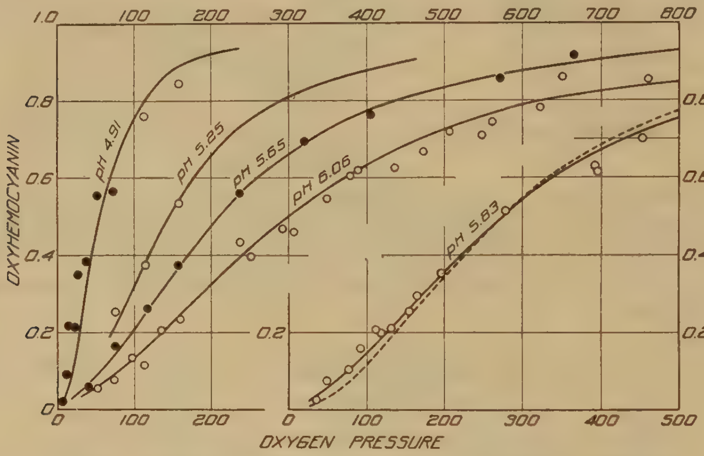

Fig. 9 Oxygen dissociation curves of serum of Homarus americanus, diluted with phosphate or acetate buffers, at acid reactions at which affinity for oxygen increases with acidity; temperature, 25°C. Ordinate, fraction of hemocyanin present as oxyhemocyanin; abscissa, oxygen pressure measured in millimeters of mercury. Curves drawn from calculations made with constants shown in table 3. Insert in lower right-hand corner shows curve calculated assuming all hemocyanin behaves as though n = 2 (broken curve); and curve calculated assuming constants shown in table 3 (continuous curve).

The curves drawn through the points in figures 7, 8, and 9 are calculated from equations 2 or 3. The constants used are presented in table 3.

Lobster blood is interesting in the present connection because its curves at suitable reactions may be described by employing a single term of equation 1. At reactions more alkaline than pH 7.7 the fourth term alone is adequate, both in the buffered and unbuffered series. At about pH 6.42 the second term alone is required and at neighboring reactions only a small quantity of the components described by the first or fourth term need be postulated.[3] The curves at intermediate reactions do not show a definite undulatory character and consequently are not particularly favorable for the demonstration of the transitional stages between the condition in which the hemocyanin behaves as though *n* equals 4 and that in which it behaves as though *n* equals 2. This is because the components into which it may be resolved are characterized by curves approximately superimposed on one another.

The blood of Busycon canaliculatum

Two preparations of Busycon blood were employed. One preparation consisted of 50 cc. fresh serum diluted with 150 cc. sea water. The oxygen dissociation curve of this preparation, which had a pH of 8.35, is illustrated in figure 3. The other preparation was made by diluting 20 cc. of serum with 50 cc. of a balanced salt solution and 10 cc. of water containing sufficient HCl or NaOH to give the mixture the requisite hydrogen ion concentration. The balanced salt solution had the following composition: NaCl 23.4 gm., KCl 0.75 gm., CaCl 2.2 gm., H_2O to 1000 cc. Curves obtained with this preparation at alkaline and acid reactions are illustrated in figures 10 and 11. The data in these figures have been fitted to curves calculated from equation 1, using the constants recorded in table 4.

Busycon serum is interesting in that it exhibits the greatest range in the complexity of the equations required to describe its oxygen equilibrium. At pH 5.79 the curve is a simple rectangular hyperbola—described by the first term of equation 1 alone—and thus similar to the purified hemocyanin of this and other species. With increasing alkalinity the curves be-

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[3] Justification for the assumption of the small fraction of the component characterized by $n = 1$ in the preparations of pH 5.65, 5.83, and 6.06 is given by the curve drawn as an insert in the lower corner of figure 9. The dotted line represents the best fit obtainable assuming $\alpha_2 = 1.0$; the solid line is drawn on the assumption that $\alpha_1 = 0.1$, $\alpha_2 = 0.9$. The data were obtained from a preparation at pH 5.83.

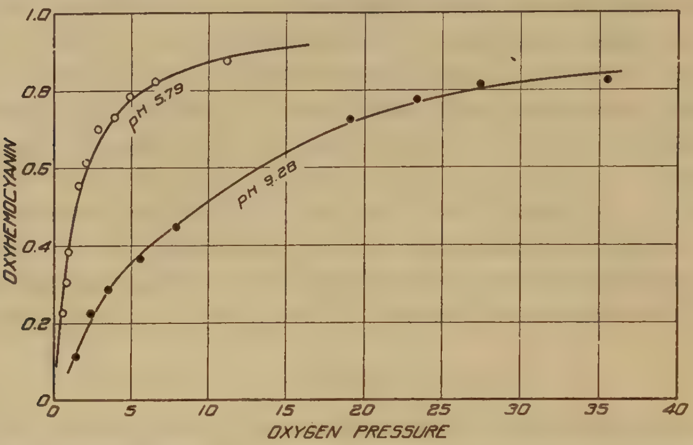


Fig. 10 Oxygen dissociation curve of serum of Busycon canaliculatum, diluted with a salt solution, at indicated pH values; temperature, 25°C. Ordinate, fraction of hemocyanin present as oxyhemocyanin; abscissa, oxygen pressure measured in millimeters of mercury. Curves calculated from constants shown in table 4.

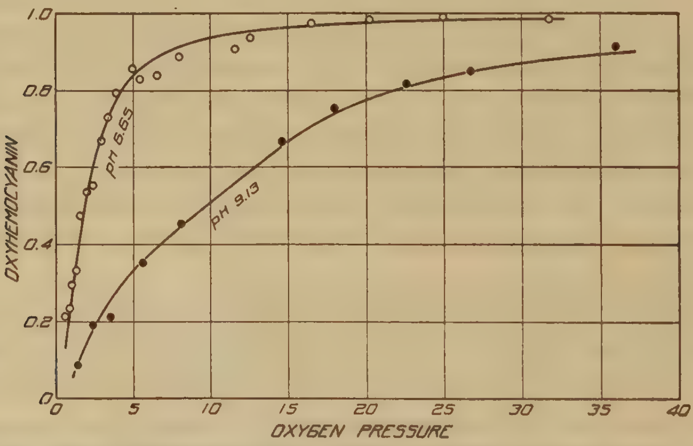


Fig. 11 Oxygen dissociation curve of serum of Busycon canaliculatum, diluted with a salt solution, at indicated pH values; temperature, 25°C. Ordinate, fraction of hemocyanin present as oxyhemocyanin; abscissa, oxygen pressure measured in millimeters of mercury. Curves calculated from constants shown in table 4.

come more complex and require the introduction of a second and third term into the descriptive equation. As in Limulus and Homarus, increasing alkalinity results, in general, in an increasing degree of interdependence between the oxygen-binding groups as evidenced by the requirement of terms of higher power in the equation. In Busycon, however, this tendency reaches a limit at pH 8.35 and in the more alkaline preparations the first term of the equation becomes increasingly important as evidenced by the values of α_1.

The blood of Loligo pealei

Oxygen dissociation curves have been measured on three different kinds of preparation of squid blood. In the first preparations the blood was diluted with sea water to which hydrochloric acid or sodium hydroxide had been added. Owing to the relatively high concentration of hemocyanin in squid blood, the serum was diluted with six volumes of sea water. Such solutions had a favorable optical density for use in the 3.3 cm. absorption cells used with the other bloods described in this paper. The optical conditions in these solutions were excellent and yielded more precise measurements than could be obtained with the bloods of other species. It was observed, however, that in the more acid solutions, at hydrogen ion concentrations at which the value of the oxygen dissociation constants varies greatly with pH, irregularities appeared which were attributed to imperfect buffering. Consequently a second series of solutions was examined in which

TABLE 4

Constants used in calculating the oxygen dissociation curves of diluted Busycon serum. Temperature, 25°C.

pH	K_1	K_2	K_4	α_1	α_2	α_4
5.79	0.7	...	....	1.0	0	0
6.65	0.7	0.25	....	0.3	0.7	0
8.35		0.07	0.18×10^{-5}	0	0.4	0.6
9.13	0.12	0.10	0.2×10^{-5}	0.5	0.2	0.3
9.28	0.10	0.15	0.2×10^{-5}	0.7	0.15	0.15

the blood was diluted with six volumes of prosphate buffer of ionic strength 0.5. These solutions yielded data of great precision. However, the shape of oxygen dissociation curves differed markedly from those obtained with blood diluted with sea water, making it apparent that the dilutant and perhaps the degree of dilution had an important effect on the nature of the oxygen equilibrium. A third series of experiments was consequently carried out on blood which had not been diluted.

Undiluted blood was equilibrated with gas mixtures containing various amounts of carbon dioxide and oxygen. The tonometers used for the determination of a single curve were all filled with the same mixture of carbon dioxide and nitrogen, after which a varying quantity of air or of oxygen was introduced into each. Thus the carbon dioxide pressure was the same in each sample. The gases were analyzed at the end of the experiment with the Haldane analyzer. The average value of the carbon dioxide determined for the individual tonometers was assigned to the entire series. The tonometers employed were similar to those previously used except that the T-piece in which the optical measurements were made was of 1 cm. length. Even with these shorter tubes the oxygenated blood was too dense to be measured with accuracy using the usual spectro-photometric technique. It was found necessary to reduce the intensity of the standard light beam by introducing a neutral filter of density 1.0 in its path, and to open the slits of the spectrophotometer to a width of 1.0 mm. With these modifications of technique the measurements obtained were distinctly less precise than those secured on diluted serum. However, they show the general character of the equilibrium in blood under essentially physiological conditions and indicate important differences between the behavior of native blood and diluted solutions. Sufficient squid blood was collected to serve for the entire series of curves. The first curve was measured on July 19, 1932. On July 30th, after the series of measurements on this lot of blood was complete, the curve was again measured under the conditions of temperature and carbon dioxide tension employed on July

19th. It was found that the properties of the blood had not changed in the interim.

The curves obtained from specimens of diluted blood are shown in figures 12 and 13. They illustrate the great change

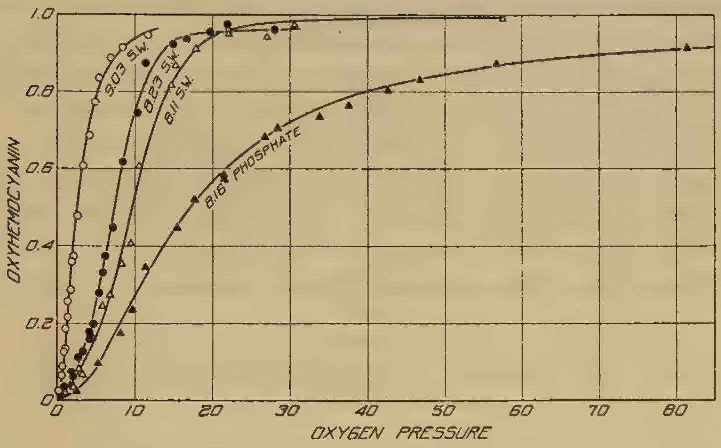


Fig. 12 Oxygen dissociation curves of serum of Loligo pealei, diluted with sea water or phosphate buffer, at alkaline reactions. Temperature, 15°C. Ordinate, fraction of hemocyanin present as oxyhemocyanin; abscissa, oxygen pressure measured in millimeters of mercury. Curves calculated from constants shown in table 5.

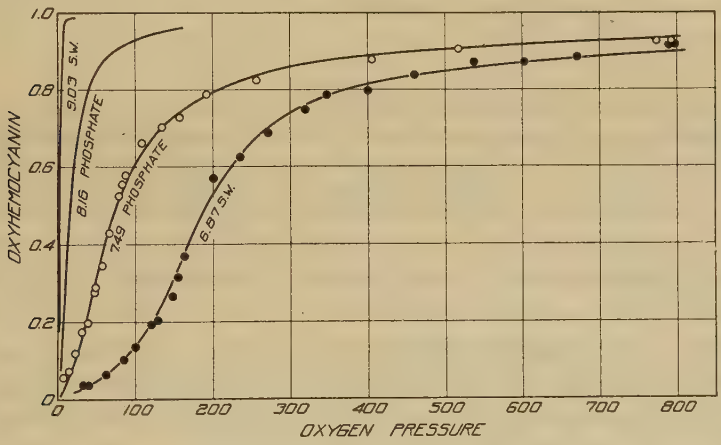


Fig. 13 Oxygen dissociation curves of serum of Loligo pealei diluted with sea water or phosphate buffer, at neutral reactions. Temperature, 15°C. Ordinate, fraction of hemocyanin present as oxyhemocyanin; abscissa, oxygen pressure measured in millimeters of mercury. Curves calculated from constants shown in table 5.

in the position of the curves which results from alteration of the hydrogen ion concentration. Simple inspection of these figures shows that the form of the curves is distinctly different when phosphate is used as a diluent.

The curves drawn through the points in figures 12 and 13 have been calculated from equations 2 and 3, using the constants recorded in table 5. The data obtained on blood diluted with phosphate is of particular interest in showing the adequacy of the equation to describe data of high precision. The constants which have been found to yield appropriate curves vary with a certain regularity. The oxygen dissociation constants K_1, K_2, and K_4 decrease as the hydrogen ion concentration diminishes. No reversal of this tendency is observed in the pH range examined. Squid blood has not been studied at the more acid reactions in which the reversal occurs in the case of crustacean hemocyanin. The variation in the values of α_1, α_2, α_4 indicates that increasing alkalinity favors the transformation of the hemocyanin into the state described by the higher terms in the equation. An exception to this is found in the specimen diluted with sea water at pH 9.03 which is described by the second power term alone. It is noteworthy that the difference in shape distinguishing the oxygen dissociation curves of blood diluted with phosphate from those of blood diluted with sea water is reflected by the fact that the former are described by the first two terms of equation 1, whereas the latter require the use of the second and fourth terms.

TABLE 5

Constants used in calculating the oxygen dissociation curves of diluted Loligo serum. Temperature, 15°

DILUENT	pH	K_1	K_2	K_4	α_1	α_2	α_4
Sea water	6.87	....	0.003	1×10^{-9}	0	0.35	0.65
	8.11	....	0.08	8×10^{-5}	0	0.2	0.8
	8.23	....	0.10	2.5×10^{-4}	0	0.2	0.8
	9.03	....	0.15	....	0	1.0	0
Phosphate buffer	7.49	0.005	0.00025	....	0.3	0.7	0
	8.16	0.03	0.004	....	0.2	0.8	0

The curves obtained from undiluted squid blood at 5°, 15°, and 25° are shown in figure 14. The constants from which the curves drawn through the experimental points were calculated are recorded in table 6. These curves differ in form from those obtained with diluted blood and are particularly interesting because most of them can be described by a single term of the equation. At 25° all the curves are described by the fourth term alone; at 15° the two more alkaline curves are so described. At 5° the two more acid solutions yielded curves adequately described by the second term of the equation. The two remaining solutions require the use of the second and fourth terms of the equation in order that the data may be adequately described. With more precise measurements the analyses of these curves may not prove so sim-

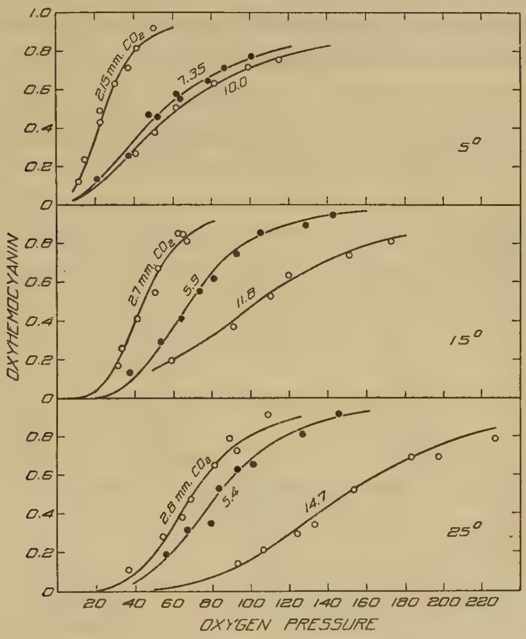


Fig. 14 Oxygen dissociation curves of undiluted squid blood at temperatures of 5°, 15°, and 25° and at indicated CO_2 pressures. Ordinate, fraction of hemocyanin present as oxyhemocyanin; abscissa, oxygen pressure measured in millimeters of mercury. Curves calculated from constants shown in table 6.

ple. The general tendency of the changes in shape and position are, however, unmistakable.

The constants show a certain uniformity in their variation similar to that noted in the other preparations. The values of K_2 and K_4 increase with increasing acidity and decrease with increasing temperature. The relative values of α_2 and α_4 indicate that higher temperatures and lower acidities favor the transformation of the hemocyanin into the form described by the higher term in the equation.

TABLE 6

Constants used in calculating the oxygen dissociation curves of undiluted Loligo serum in the presence of CO_2

TEMPERATURE	CARBON DIOXIDE PRESSURE	pH[1]	K_2	K_4	α_2	α_4
Degrees C.	*Mm. Hg.*					
25	14.7	6.96	......	2×10^{-9}	0.	1.0
	5.4	7.15	......	2.1×10^{-8}	0.	1.0
	2.8	7.28	......	4.2×10^{-8}	0.	1.0
15	11.8	7.02	0.0002	5×10^{-9}	0.4	0.6
	5.9	7.14	......	4.4×10^{-8}	0.	1.0
	2.7	7.29	......	2.5×10^{-7}	0.	1.0
5	10.0	7.05	0.00025	....	1.0	0
	7.35	7.10	0.00032	....	1.0	0
	2.15	7.34	0.002	2×10^{-6}	0.5	0.5

[1] pH values are calculated from the CO_2 dissociation curve of squid blood described by Redfield, Coolidge, and Hurd ('26). They must be considered as only approximate.

DISCUSSION

The equations developed to describe the equilibrium between hemocyanin and oxygen have proved adequate in a large number of cases in which hemocyanins of divergent properties are studied under a wide range of conditions. The test of an hypothesis by 'curve fitting' has at best, however, a limited value, since it must always be recognized that alternate hypotheses may yield equally satisfactory formulations. Not only is the physical meaning of the various constants in the equation open to doubt, but one may question whether the constants really represent any discrete physical phenomena. Quite possibly they are accidental mathematical abstractions which happen to yield curves which describe the experimental results.

Whether the constants represent underlying physical phenomena or not may be tested by examining the way in which they vary with the conditions under which their particular values are determined. If this variation has an orderly character, one must accept the probability that this order is a reflection of some order in the behavior of the underlying physical phenomena. If such orderly behavior in the constants can be related to the behavior of known physical phenomena, belief in the physical significance of the constants is greatly strengthened.

The variation in the constants used to characterize the oxygen dissociation curves of hemocyanin, under varying conditions of hydrogen ion concentration and temperature, has been reviewed from this point of view.

The values of α_1, α_2, *and* α_4

These constants represent the various fractions of the total hemocyanin existing in the forms in which $n = 1$, 2, and 4. Variation in their values represents the process whereby the hemocyanin changes in the degree to which the prosthetic groups are assumed to become interdependent in combining with oxygen. In terms of the Hill ('10) theory as originally presented, it would represent the changes in degree of aggregation of the respiratory protein. In view of existing data on the molecular weight of hemocyanin (Svedberg and Eriksson, '32), which is such that each molecule may combine with 20 to 40 molecules of oxygen, it seems more plausible to think of it as representing changes in the association of various prosthetic groups on a single molecule resulting from changes in the configuration of the molecule itself.

From the data presented in the tables the values of α_1, α_2, and α_4 have been selected in such cases as yield a series sufficient to show how one form changes to another as a function of pH. These values are plotted in figure 15. It may be seen that, in general, a transition occurs from a form in which n is small to a form in which n is twice as great as one passes from acid to alkaline solution. In each case it appears—so far as the data are adequate to show it—that this transition occurs close to pH 7 and that it occurs quite abruptly. A similar transition in the opposite direction (from forms with high to low values for n) occurs at alkaline reactions in the case of Busycon and Loligo. The data of Stedman and Stedman ('26 a) suggest a similar tendency in Homarus hemocyanin at reactions more alkaline than we have explored.

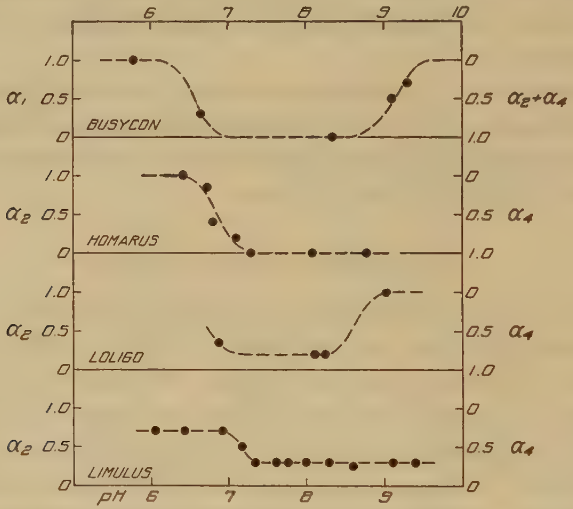


Fig. 15 The variation in values of α_1, α_2, and α_4 of different hemocyanins, as a function of pH.

The nature of the variation of the α values with pH is similar to that observed in a large number of phenomena in which the properties of a substance are varied through the reactions of acidic or basic groups. These facts support the view that the α-constants represent real physical phenomena and suggest that these phenomena depend upon a particular type of chemical reaction. On this view it is possible to ascribe definite pK values to the acid base equilibria involved and to state, at least in the case of the change leading to the formation of reactive groups characterized by higher values of n, that the pK values are nearly the same in the case of the several kinds of hemocyanin.

Svedberg and Eriksson ('32) have demonstrated by the ultra centrifugal method that the hemocyanin of the octopus, both in purified solutions and in the native blood, consists of two components, one of high molecular weight and one of lower molecular weight. The two components are not considered to be two different proteins, but represent two different kinds of aggregation in equilibrium. The relative proportions of the two components varies with pH in a manner not dissimilar to that of the components designated by α_1, α_2, and α_4 in the present paper. It seems quite probable that the phenomena described by Svedberg and Eriksson and those discussed in this paper may have a common physical basis.

Temperature alters the values of α_2 and α_4 in the case of Limulus and Loligo serum. In both cases a rise in temperature increases the fraction of hemocyanin in the form designated by α_4. The two forms may be considered to be in equilibrium with one another and one may consequently assign an equilibrium constant to the reaction. Since α_2 and α_4 measure relative concentrations, one may write

$$\frac{\alpha_4}{\alpha_2} = K_a$$

The variation of K_a with temperature follows the van't Hoff isochore between 15° and 44°, as shown by the values for Limulus serum plotted in figure 16. The value at 5° is not concordant, but at this temperature the curve is complex and the accuracy of the analysis is not great. One may conclude that heat is absorbed in changing the form designated by α_2 into the form designated by α_4. The heat absorbed in the transformation is the same at all temperatures and for all proportions of the components and amounts to approximately 10,000 calories. Thus, the values of α_2 and α_4 vary with temperature in an orderly fashion, such as would be expected if they represent concentrations of entities in an equilibrium with one another, which equilibrium is determined by the heat exchange involved in the transformation of one into the other.

The values of K_1, K_2, and K_4

The way in which the oxygen dissociation constants of the several forms of hemocyanin vary with hydrogen ion concentration is indicated in figure 17, in which are plotted such data as are sufficiently complete to be of interest. It is found that the hemocyanin of the four species may be divided into two categories. The magnitude of the constants and the way in which they vary in respect to pH is similar for the species in each group. These characteristics of the constants of one group are very different from those of the other. The two groups are plotted on separate scales in figure 17. The left-hand curves represent the values of K_1, K_2, and K_4 for Homarus and Loligo; the right-hand, the values for Busycon and Limulus. These similarities and differences in the case of the two groups are so striking that it seems safe to predict that some definite difference in the structure of the prosthetic groups of Homarus and Loligo hemocyanins will distinguish these proteins from the hemocyanins of Busycon and Limulus.

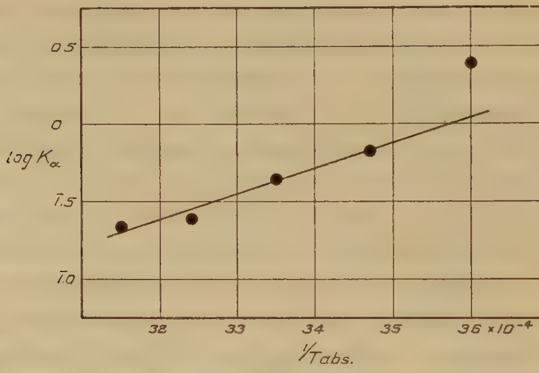


Fig. 16 Variation of the constant, K_a, after defining the equilibrium between the forms of Limulus hemocyanin measured by a_3 and a_4 at various temperatures. Ordinate, logarithm of the value of K_a; abscissa reciprocal of absolute temperature. Data from table 2.

It has been shown by Redfield and Ingalls ('32) that in the case of the purified hemocyanin of Busycon the oxygen dissociation constant, K_1, varied with hydrogen ion concentration in accordance with the equation

$$K_R = \frac{(H)^2 (K_1 - K_a)}{(K_s - K_1)} \tag{4}$$

in which K_a is the oxygen dissociation constant at maximal acidity and K_s is the oxygen dissociation constant at minimal acidity, and K_R is the acid dissociation constant of the reaction responsible for the change in oxygen affinity of the hemocyanin. This expression was originally derived by Ferry and Green ('29) to describe the variation of the oxygen dissociation constants of hemoglobin with acidity.

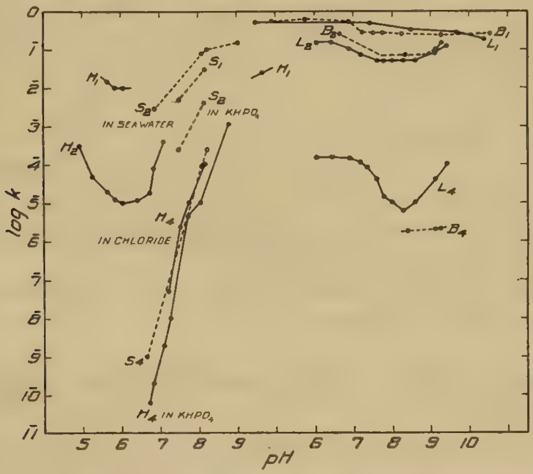
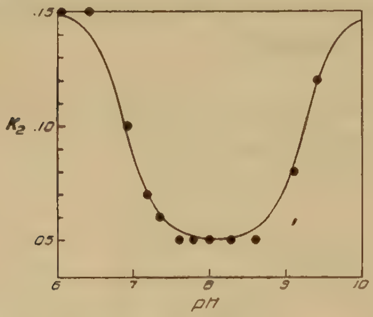


Fig. 17 Relation of values of the oxygen equilibrium constants of the various forms of the hemocyanin of different species to hydrogen ion concentration. Ordinate, logarithms of the values of K_1, K_2, and K_4; abscissa, pH values. H_1, values of K_1 for serum of Homarus americanus (the value at pH 9.6 is the Stedmans' value for H. vulgaris). H_2, values of K_2 for serum of Homarus americanus. H_4, values of K_4 for serum of Homarus americanus. S_1, values of K_1 for serum of the squid, Loligo pealei, in phosphate buffer. S_2, values of K_2 for serum of squid, in sea water and in phosphate buffer. S_4, values of K_4 for serum of squid in phosphate buffer. B_1, values of K_1 for Busycon hemocyanin after Redfield and Ingalls, '32. B_2, values of K_2 for Busycon serum. B_4, values of K_4 for Busycon serum. L_1, values of K_1 for Limulus hemocyanin. (After Redfield, '30). L_2, values of K_2 for Limulus serum. L_4, values of K_4 for Limulus serum.

The values of K_1, K_2, and K_4 recorded in figure 17 all vary with hydrogen ion concentration in a way compatible at least with this equation. Only in the case of Limulus serum is the data sufficiently complete to warrant critical examination. These data are plotted in figures 18 and 19. The curves drawn through the values of K_2 and K_4 between their maximal (acid) and minimal values are computed from equation 4. Beyond the minimum symmetrical curves have been drawn through the values calculated for the more alkaline solutions. It appears that the values of K_2 and K_4 for Limulus serum vary with pH in a manner characteristic of the oxygen dissociation constants derived for hemoglobin, and simpler hemocyanin systems.



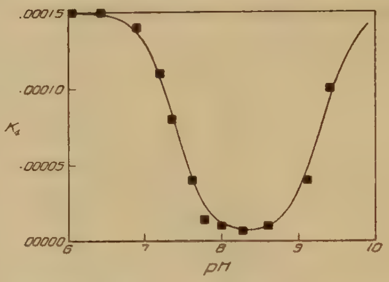


Fig. 18 Relation of K_2 for Limulus serum and pH. The curve is calculated from equation 4. Data are recorded in table 1.

Fig. 19 Relation of K_4 for Limulus serum and pH. The curve is calculated from equation 4. Data are recorded in table 1.

The effect of temperature upon the equilibrium of hemocyanin with oxygen has been studied by Hogben ('26), Hogben and Pinhey ('26, '27), and Brown ('33). Brown showed that the oxygen dissociation constant (K_I and K_{II}) of purified Limulus and Busycon hemocyanin varied with temperature (T_I and T_{II}) in accordance with the equation

$$q = \frac{RT_I T_{II}}{(T_I - T_{II})} \ln \frac{K_I}{K_{II}} \qquad (5)$$

and that $q = 14{,}800$ calories per gram molecule of oxygen combined in the case of Limulus hemocyanin, and $q = 16{,}000$

in the case of Busycon hemocyanin. We have attempted to treat the constants for Limulus serum recorded in table 2 in accordance with this relation. Plotting the values of K_2 and K_4 in accordance with this expression in figure 20, it appears that the values of K_2 follow the linear requirement of equation 5. With regard to K_4, the slope of the curve relating its logarithm to the absolute temperature is not constant, there being a threefold increase between 15° and 25°. This feature is unsatisfactory, and we believe it indicates that the assumptions underlying the analysis of the data may not be completely adequate.

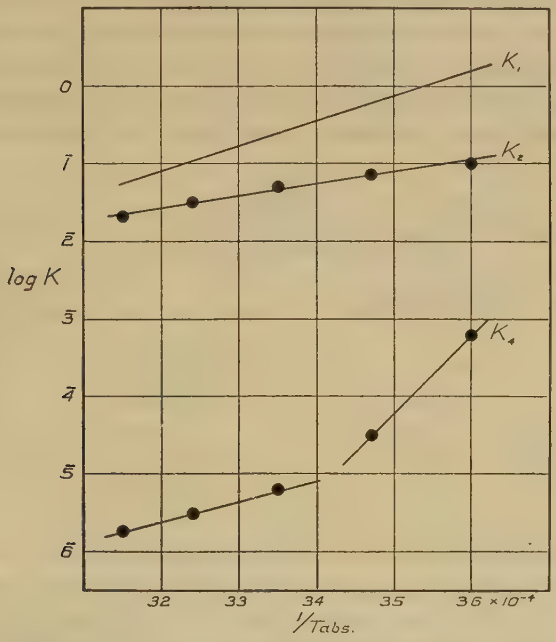


Fig. 20 Variation of the values of the oxygen dissociation constants (K_1, K_2, K_4) of Limulus hemocyanin with temperature. Ordinate, logarithm of the value of K; abscissa, reciprocal of absolute temperature. Data for K_2 and K_4 from table 2; for K_1 from Brown ('33).

K_2 and K_4 are equilibrium constants supposedly descriptive of processes in which 2 and 4 molecules of oxygen, respectively, react with the hemocyanin. In consequence q must represent the heat of union of 2 and of 4 molecules of oxygen with the respective forms of hemocyanin. We may consequently determine the value of the heat developed in the union of 1 molecule of oxygen with the protein by dividing q by n, the number of oxygen molecules entering into the reaction. The results obtained are recorded in table 7.

The values of the constants derived from the application of equation 1 to data for oxygen dissociation curves at a variety of temperatures and hydrogen ion concentrations vary in an orderly manner, characteristic of known physical phenomena. Some justification is afforded for the belief that real physical phenomena underlie these constants and that the theory from which the equation is derived describes at least vaguely the nature of the factors responsible for the complexity of the oxygen dissociation curve of bloods containing hemocyanin.

TABLE 7

Heat of oxygenation of Limulus hemocyanin calculated from the oxygen equilibrium constants K_1, K_2, and K_4

EQUILIBRIUM CONSTANT	TEMPERATURE RANGE	n	q	q/n
	Degrees C.		*Calories per n mols oxygen*	*Calories per mol oxygen*
K_1	5–40	1	14,800	14,800
K_2	5–44	2	9,120	4,560
K_4	25–44	4	12,000	3,000
	5–15	4	36,900	9,225

SUMMARY

1. An expression, based on the mass law and derived from the assumptions underlying the equation of Hill, is developed to describe the equilibrium between hemocyanin and oxygen.

2. It is assumed that the hemocyanin may occur in the blood as a mixture of two or more components, each of which reacts with oxygen in accordance with Hill's equation, but that the components are each characterized by forming a stable oxidation product with a different number of oxygen molecules.

3. The oxygen dissociation curves of the blood of the horseshoe crab, Limulus polyphemus, the lobster, Homarus americanus, the conch, Busycon canaliculatum, and the squid, Loligo pealei, can be described by this equation under a great variety of conditions of hydrogen ion concentration and temperature.

4. Under suitable conditions, solutions may be obtained which appear to contain the various components assumed by the complete expression, in isolated form.

5. The values of the oxygen equilibrium constants derived for the various components and the constants expressing the relative proportions in which they occur vary in an orderly manner with hydrogen ion concentration and temperature.

6. On the basis of the magnitude of the oxygen dissociation constants of the various components and the way in which they vary with hydrogen ion concentration, the hemocyanins may be divided into two categories which appear to have distinctly different prosthetic groups.

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Contribution No. 17

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MARINE BACTERIA AND THEIR RÔLE IN THE CYCLE OF LIFE IN THE SEA

I. DECOMPOSITION OF MARINE PLANT AND ANIMAL RESIDUES BY BACTERIA [1]

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The synthesis of organic matter in the sea through the agency of the chlorophyll-bearing plants, ranging from the smallest diatoms to the largest algæ, consists in the assimilation of the carbon as CO_2, of the nitrogen as nitrate, and probably to a lesser extent as ammonia and nitrite, of the phosphorus as phosphate, and of other elements in lower concentrations. Before these elements can be returned to circulation, after the plants as well as the animals which fed partly upon them die, the complex organic substances have to be decomposed through the agency of bacteria. If one is to judge by analogy with the decomposition processes which take place on land, the liberation of the elements in a mineralized form does not represent a simple process, but rather a chain of processes. The rate of liberation of the elements by bacterial action depends primarily upon the chemical composition of the materials undergoing decomposition and upon the organisms active in the decomposition processes.

The following investigations were undertaken for the purpose of determining to what extent the chemical composition of marine residues of plant and animal origin influences the rate of their decomposition by marine bacteria and the rate of liberation of the important elements, especially nitrogen, in an available form. It was further essential to obtain light upon the mechanism of decomposition of some of the chemical constituents of the marine residues by specific members of the bacterial population of the sea. These investigations can be classified, therefore, under three distinct headings, namely: (1) the chemical composition of marine zoöplankton and of certain marine algæ, (2) the decomposition of the plankton and algal material in sea water and in marine mud by bacteria, (3) the decomposition of certain algal constituents by specific bacteria isolated from the sea.

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[1] Contribution No. 17 of the Woods Hole Oceanographic Institution and Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Soil Microbiology.

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MATERIALS USED AND THEIR CHEMICAL COMPOSITION

Two types of organic matter of marine origin were used in this investigation, one representing zoöplankton and one algal material. The zoöplankton was collected on July 6, 1932, in Buzzard's Bay, by means of an ordinary scrim plankton net. As soon as brought to the laboratory, the excess liquid was pressed out through cheesecloth and through a fine silk net, and plankton then dried in a current of warm air. The plankton consisted largely of copepods (*Centropages hamatus* and *C. typicus*), a few isopods, decapods, *Sagitta elegans,* fragments of hydroids, and fish eggs. The liquid pressed out by ordinary hand pressure from the original plankton was examined and found to consist largely of bacteria and some colloidal material.

The air-dry plankton contained 6.7 per cent moisture. This material was subjected to analysis and the results calculated on a dry basis. The total dry plankton was found to consist of 7.88 per cent nitrogen, 28.1 per cent ash, of which 15.1 per cent was sea salt as calculated from the chlorine content. The complete proximate analysis (22) of the plankton is given in Table I.

TABLE I

Proximate analysis of zoöplankton. Dry, salt-free basis.

Chemical constituent	Percentage
Ether-soluble fraction *	10.1
Alcohol-soluble, non-nitrogenous *	4.8
Cold water-soluble *	3.1
Hot water-soluble *	2.0
Chitin	5.8
Protein	55.7
Cellulose	2.4
Lignin (?)	2.8
Ash, salt-free	13.0
Total accounted for	99.7

* Ash and nitrogen-free basis.

The chitin content was determined on a separate sample, by repeated treatment with hot dilute hydrochloric acid (2 per cent) and hot 12 per cent KOH solution, then washing with alcohol and ether. The nitrogen of the chitin was subtracted from the total nitrogen of the material and the rest calculated as protein; using the factor 6.25. The cellulose content was calculated from the amount of sugar produced on hydrolysis of the plankton residue (after extraction with hot dilute acid) with 80 per cent sulfuric acid in the cold, diluting with 15 volumes of water and heating in order to complete the hydrolysis. The total sugar formed on hydrolysis was corrected for the reducing sugar produced from the chitin and calculated as cellulose, using the factor 0.9. The cold water extract of the plankton was free from reducing sugar, while the hot water and the hot dilute acid extracts were free from glycogen and hemicellulose. Just as in the case of the alcohol extract, the cold and hot water-soluble fractions were corrected for ash and nitrogenous constituents.

The danger of using alcohol for the preservation of the plankton is brought out by the fact that when treated with 95 per cent ethyl alcohol, the total plankton gave 24.7 per cent alcohol-soluble material, of which 23.2 per cent was organic matter. The low figure reported in the table for the alcohol-soluble fraction is due to the fact that in the complete analysis the dry plankton was first extracted with ether, while the total alcohol-soluble portion was corrected for ash and protein, so as to avoid duplicating the same constituent in the final analysis; since a large part of the ether-soluble material and some of the nitrogenous constituents are also soluble in alcohol, the corrections made resulted in reducing the concentration of the remaining alcohol-soluble fraction.

The results of this analysis are quite in accord with similar analyses made by Brandt (2), who employed, however, the methods commonly used in foodstuff analysis, whereby the chemical constituents were reported as crude fat, protein, carbohydrate, crude fiber, and nitrogen-free extractives.

It is important to call attention, in connection with this analysis, to the high content of organic nitrogenous constituents in the zoöplankton as well as of fatty substances and of chitin, and to the low content of carbohydrates. The amount reported as cellulose is so small that one may be justified in questioning whether this represents true cellulose or other complexes, which give, in addition to chitin, reducing substances on hydrolysis with concentrated acid; the presence of a small amount of cellulose may also be due to certain constituents of plant origin. The same is true of the lignin; the concentration of this complex is so small that its origin in the plankton may still be considered as an open question; it may actually represent a certain polyuronide not readily hydrolyzed by 80 per cent sulfuric acid in the cold (14).

As sources of algal material, *Fucus vesiculosus, F. platycarpus,* and *Ulva lactuca* were selected. In the case of the *Fucus,* the material was brought to the laboratory as soon as collected, cleaned thoroughly by hand to remove extraneous matter, washed 2–3 times with tap water, then with distilled water to remove the excess of salt, and dried at about 80–90° C. In the case of the *Ulva,* however, the material was dried directly, without preliminary washing. The results of the analysis of these three algal materials are reported in Table II.

The cold water-soluble fraction of the *Fucus* contained 1 to 2.5 per cent reducing sugar, on the basis of the total dry material. The hot water-soluble fraction, when hydrolyzed with dilute acid, gave 2.8 to 3.5 per cent reducing sugar, pointing to the presence in this fraction of complex carbohydrates. After the hot water extraction, the material was treated with 2 per cent hydrochloric acid, at 100° C., for 5 hours; the extract was removed by filtration, neutralized and analysed for reducing sugar; the results obtained are reported as "hemicelluloses." However, this figure does not represent the total hemicellulose, including both true polysaccharides and polyuronides; when separate samples were analyzed for uronic acid and for total pentosan (furfuraldehyde method), considerably larger figures were obtained than those given under "hemicelluloses." This is due to the fact that the furfuraldehyde formed on treatment of the material with 12 per cent hydrochloric acid may be due to substances consisting not only of pentoses derived from pentosans but also of uronic acid complexes forming polyuronides. The latter change on hydrolysis only incompletely to reducing sugar. The three sets of results for the carbohydrate groups, namely the reducing sugar formed on hydrolysis with dilute acid, the pentosan, and the uronic acid anhydride, overlap and render it, therefore, impossible to make a final addi-

TABLE II

Chemical composition of three marine algæ. Dry, salt-free basis.

Organism	*Fucus vesiculosus*	*F. platycarpus*	*Ulva lactuca*
	per cent	*per cent*	*per cent*
Chemical constituent:			
Ether-soluble fraction	2.3	2.3	0.8
Alcohol-soluble fraction	6.7	8.7	10.6
Cold water-soluble organic matter	13.6	12.1	17.9
Hot water-soluble organic matter	7.8	8.1	10.7
"Hemicelluloses," as reducing sugars	11.2	11.8	11.0
Uronic acid anhydride	27.5	16.1	6.2
"Pentosan," by furfuraldehyde method	28.7	24.5	31.4
"Cellulose," as reducing sugar	4.3	5.4	6.2
"Lignin," or complexes not acted upon by 80 per cent H_2SO_4 in the cold *	5.6	6.2	2.9
Protein	5.4	4.7	12.4
Ash	13.9	14.6	13.6

* This preparation not acted upon by cold 80 per cent H_2SO_4 and boiling 5 per cent H_2SO_4 is not lignin, but a largely carbohydrate-like complex. It is of interest to note that Butler (4) reported the presence of 5.21 per cent "crude fiber" in *F. vesiculosus* and that Schmidt (23) found that 40 per cent of the polyglycuronic acid in *F. serratus* was insoluble in cold 80 per cent H_2SO_4.

tion of the results. The fractions reported as "cellulose" and "lignin" do not necessarily represent the corresponding complexes in higher plants, for considerations similar to those presented above for these constituents in the zoöplankton.

The results of the analyses of the algal materials are also in line with those found by others. These algæ are usually reported (3) to contain from 1.37 to 3.71 per cent nitrogen, 1.50 to 5.21 per cent crude fiber, pointing to a low cellulose and lignin content, and 15.7 to 33.2 per cent ash. The organic complexes are made up largely of carbohydrates, which could be classified with the hemicelluloses, including both true saccharides and polyuronides (8). Although a few specific carbohydrates have been definitely demonstrated (8, 10) in algal material, such as mannitol, arabinose, galactose and methyl pentose or fucose, most of the constituents are still imperfectly described under such names as algin, laminarin, fucoidin, fucin, etc. Some of these complexes represent polyuronides, as in the case of the polymer of mannuronic acid, which is readily isolated from *Fucus* by dilute alkali solutions (1, 11).

Decomposition of Zoöplankton by Marine Bacteria

Our knowledge of the function of bacteria in decomposing the plant and animal residues in the sea is still very limited, the available information being largely based upon speculation rather than upon experimental evidence. It was early recognized that the bacteria active in the decomposition processes in the sea also synthesize new organic complexes in the form of bacterial cell substance. Vernon (17), for example, reported that the largest amount of purification of ocean waters is carried on through bacterial agency; when sea water was kept in the darkness the bacteria removed a large part of the ammonia in the water and synthesized considerable slime; larvæ grown in this water were larger than normal, which led Vernon to conclude that the bacterial cells serve as nutrients for these larvæ. The energy necessary for this bacterial synthesis is no doubt derived from the dissolved organic substances in the water, which have been found (12) to range from 3 mg. of carbon per liter of water in the Baltic Sea to 11–14 mg. in the Kiel Fjord. However, Moore (9) concluded that only a maximum of 1 mg. of organic matter is dissolved in one liter of water. According to Gran and Ruud (5) and Krogh (7), the organic matter in solution is greatly in excess over that present as plankton; this organic matter was looked upon as mainly a waste product, which is not readily available for animal nutrition, and only to a limited extent to bacterial development. Korinek (6) has shown that when fresh water bacteria are placed in the sea they are unable to attack algal residues, while marine bacteria are able to bring about active decomposition of the algal material; however, no quantitative measurements were made. The decomposition of marine algæ added to the soil brought out the fact (15) that the larger the nitrogen content of the algæ the greater is the amount liberated as ammonia during decomposition.

In the following investigations on the decomposition of marine plant and animal residues by bacteria, not only was there an attempt made to study the rate of decomposition and the liberation of nitrogen as ammonia, but also to establish definite relationships between the composition of the substrate and the rate and nature of its decomposition.

For the study of the decomposition of the zoöplankton, 150-cc. portions of fresh sea water were placed in a series of 300-cc. long-necked flasks; another series of flasks received 100-gram portions of fresh marine mud (containing about 50 per cent moisture) and an additional 50 cc. of fresh sea water. Some of the flasks were left as controls, while some received one-gram portions of the air-dry plankton. All the flasks were connected, in the laboratory (16–20° C.), with an aëration apparatus (21), and aërated daily for about one hour. The air, first freed from CO_2, was made to bubble through the liquid of the culture or through the surface layer of water, in the case of the mud cultures, and was then passed through tubes containing standard $Ba(OH)_2$ solution, where the CO_2 liberated in the process of decomposition was absorbed. The experiment was allowed to proceed for 19 days.

TABLE III

Decomposition of zoöplankton by bacteria in sea water and in marine mud *

Medium used	Amount of plankton added	CO_2 liberated		NH_3 liberated
		total	above control	
		mgm. C.	*mgm. C.*	*mgm. N.*
Water.........	0	5.6	—	0.10
Mud...........	0	3.2	—	0.25
Water.........	1 gram	69.7	64.1	40.30
Mud...........	1 gram	103.9	100.7	38.50

* Period of decomposition—19 days.

At the end of the decomposition period, 2-cc. portions of concentrated H_2SO_4 were added to each flask and the cultures thoroughly aërated for about 1 to 2 hours, so as to liberate the CO_2 absorbed in the water. The flasks were then disconnected and analyzed for ammonia, by distillation with MgO; in the case of the mud cultures, the material was first extracted with N/1 KCl solution, and the ammonia determined in the extract. The cultures contained no nitrite or nitrate. The results of the decomposition of the zoöplankton in the sea water and in the mud are given in Table III, while the course of evolution of CO_2 is illustrated graphically in Figs. 1 and 2. The precipitous rise in the amount of CO_2 at the end of the decomposition period is due to the liberation by the final acid treatment of the CO_2 present in the medium as carbonate and bicarbonate.

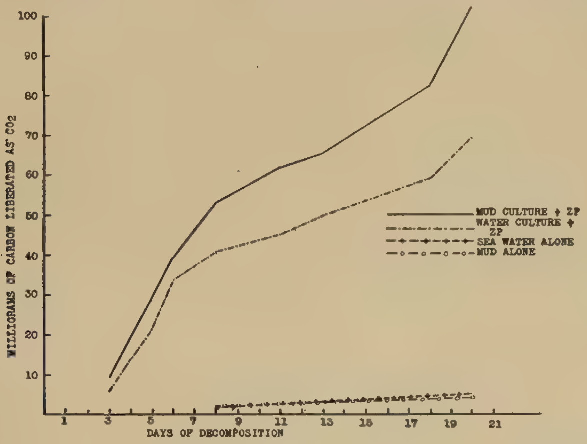

FIG. 1. Accumulated evolution of CO_2 in the course of decomposition of zoöplankton in sea water and in marine mud cultures.

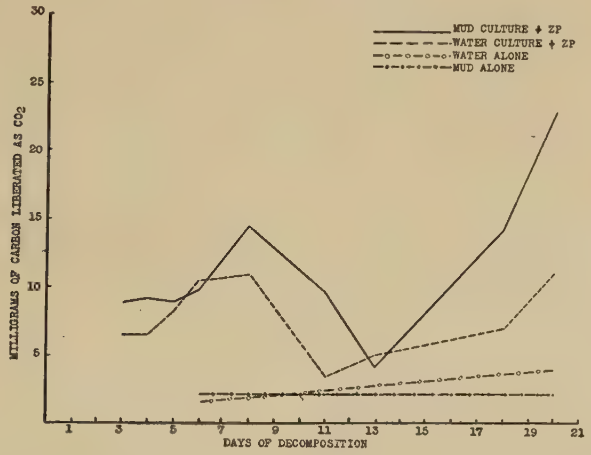

FIG. 2. Daily evolution of CO_2 in the decomposition of zoöplankton in sea water and in marine mud cultures.

The amount of CO_2 liberated from the plankton in the mud as a medium is much greater than in the sea water, while the amount of nitrogen liberated as ammonia is practically the same in both media. Since one gram of the air-dry plankton contained 625 milligrams of organic matter (allowing for moisture and total ash), with about 300 milligrams of total carbon, the liberation in the mud medium of 100 milligrams of carbon as CO_2 within 19 days indicates a very rapid decomposition of the plankton. However, more than a half of the total nitrogen in the plankton has been liberated as ammonia in the same culture and during the same period of decomposition; this points to two definite alternatives: 1. The various chemical constituents of the plankton are not all decomposed at the same rate. 2. More of the plankton is actually decomposed than one is able to measure by the CO_2 evolution, since considerable synthesis of bacterial cell substance takes place in the process of decomposition. The first suggestion is borne out by an examination of the curves in Fig. 2. Although the rate of decomposition had come to a minimum in 11 days, in the case of the sea water medium, and in 13 days, in the case of the mud medium, further decomposition began to take place after that date.

TABLE IV

Decomposition of marine algæ by bacteria in sea water and in marine mud *

Medium used	*Ulva lactuca*			*Fucus vesiculosus*		
	CO_2 liberated		NH_3-N liberated	CO_2 liberated		NH_3-N liberated
	total	above control †		total	above control †	
	mgm. C.	*mgm. C.*	*mgm. N.*	*mgm. C.*	*mgm. C.*	*mgm. N.*
Water........	89.0	83.4	5.2	16.0	10.4	0.4
Mud..........	105.0	101.8	4.1	89.0	85.8	—

* 1 gram of air-dry material used; 26 days incubation for *Ulva* and 28 days for *Fucus*.

† Water and mud controls were the same as in Table III.

These results suggest the following possibility: some of the constituents of the plankton, probably the proteins, are undergoing active decomposition during an early stage; this is later followed by the decomposition of other constituents of the plankton, probably the chitins and the fatty substances. The specific bacteria capable of attacking these two complexes are probably more active in the mud than in the water. Their decomposition of these substances which are low in nitrogen or are totally free from nitrogen will not only not result in any ammonia liberation but may actually result in ammonia consumption by bacteria for cell synthesis. These two considerations may fully account for the greater decomposition of the plankton in the mud than in the water, as measured by the CO_2 evolution, not accompanied, however, by greater ammonia liberation.

Decomposition of Marine Algæ by Bacteria

The decomposition of the algal material by bacteria was carried out in a manner similar to that of the plankton. The period of decomposition was 26 days for the *Ulva* and 28 days for the *Fucus*. One-gram quantities of the air-dry material of *F. vesiculosus* and *U. lactuca* were added to the flasks containing the water as a medium or the mud and water; the cultures were aërated daily.

The results presented in Table IV and Fig. 3 show that the *Ulva* was decomposed by the bacteria in the water and in the mud more actively than the *Fucus*. In the case of the *Ulva* there was very little difference in the rate of decomposition in the water or in the mud; the *Fucus*, however, showed a very marked difference, since a considerably greater amount of CO_2 was liberated in the mud than in the water cultures. About 4 to 5 milligrams of ammonia-nitrogen were accumulated in the decomposition of the *Ulva*, while there was no ammonia at all or only a mere trace in the *Fucus* cultures. The nitrogen content of the two algæ offers the explanation for this difference. *Ulva* contained 2.56 per cent nitrogen on a total dry, ash and salt-free basis; *Fucus*, however, contained only 1.01 per cent nitrogen, on an ash and salt-free basis.

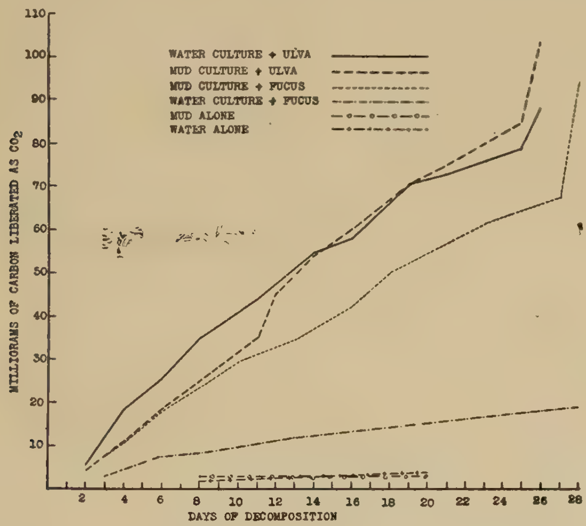

Fig. 3. Course of evolution of CO_2 in the decomposition of marine algæ in sea water and in marine mud.

The difference in the nitrogen content of the two materials has an important effect upon the rapidity and nature of their decomposition. It has been found (23) in the decomposition of plant residues in soils and in composts that if the material contains 1.7 per cent nitrogen, the latter is just sufficient for the active decomposition of the plant substance, without any nitrogen being liberated as ammonia and without any added nitrogen required by the microörganisms. When the plant material contains more than 1.7 per cent nitrogen, the excess will be liberated as ammonia. However, when the nitrogen content is less than 1.7 per cent, some inorganic or available form of nitrogen must be added to enable the bacteria and the fungi to decompose the plant material.

The same law seems to hold true also for the decomposition of algal residues in the sea. There was more than enough nitrogen in the *Ulva* to bring about its rapid decomposition by the bacteria, as shown by the fact that out of 1 gram of the air-dry material, containing 590 milligrams of organic matter with about 265 milligrams carbon, there was liberated nearly 100 milligrams carbon as CO_2 or about 40 per cent of the total, both in the water and in the mud cultures. The *Fucus*, however, containing 827 milligrams of organic matter and about 372 milligrams of carbon in 1 gram of the air-dry material, did not contain sufficient nitrogen for its rapid decomposition by the bacteria.

TABLE V

Influence of addition of available nitrogen upon the decomposition of Fucus vesiculosus in sea water *

Treatment	CO_2 liberation		Inorganic nitrogen left	Inorganic nitrogen disappeared	Numbers of bacteria in 1 cc. of culture
	total	due to added nitrogen			
	mgm. C.	*mgm. C.*	*mgm. N.*	*mgm. N.*	
2 grams Fucus alone	18.9	—	0	0	14,400,000
2 grams Fucus + 50 mgm. $NaNO_3$	69.5	50.6	0	8.0	70,600,000
2 grams Fucus + 300 mgm. $NaNO_3$	54.7	35.8	0.5	47.5	31,000,000
2 grams Fucus + 50 mgm. $(NH_4)_2SO_4$	47.2	28.3	4.6	5.9	28,600,000

* 2-gram portions of air-dry *Fucus* in 150 cc. of water; decomposition, 28 days.

The greater decomposition of the *Fucus* in the mud than in the sea water may be due to two factors: 1. The organic matter in the mud undergoes some decomposition and the amount of nitrogen liberated in this process may be sufficient for the need of the bacteria, thus enabling them to bring about considerably greater decomposition of the *Fucus*. 2. There is also a possibility that the mud may have a greater abundance of specific bacteria capable of attacking certain specific carbohydrates in the *Fucus*, while the water may be lacking in such bacteria.

In order to study further the factors that are concerned in the decomposition of the nitrogen-poor *Fucus* by marine bacteria, the influence of available nitrogen, both as ammonia and nitrate, has been determined. A series of cultures were prepared using sea water as the medium. All the cultures received 2-gram portions of air-dry, ground material of *Fucus vesiculosus*. The course of decomposition was followed by measuring the evolution of CO_2, in a manner similar to that outlined above. At the end of the incubation period (28 days), the residual nitrogen left as nitrate or as ammonia was determined. The results of this experiment are given in Table V and in Fig. 4.

When no available nitrogen was added to the medium, only a limited amount of *Fucus* underwent decomposition. The addition of a small amount of nitrogen, in the form of an inorganic salt, brought about considerably greater decomposition, the nitrate nitrogen having a more favorable effect than the ammonium salt. An excess of nitrate, however, was less effective than a lower concentration. Where the lower amount of nitrate was added to the cultures, all the nitrogen was consumed by the bacteria bringing about the decomposition of the *Fucus*,

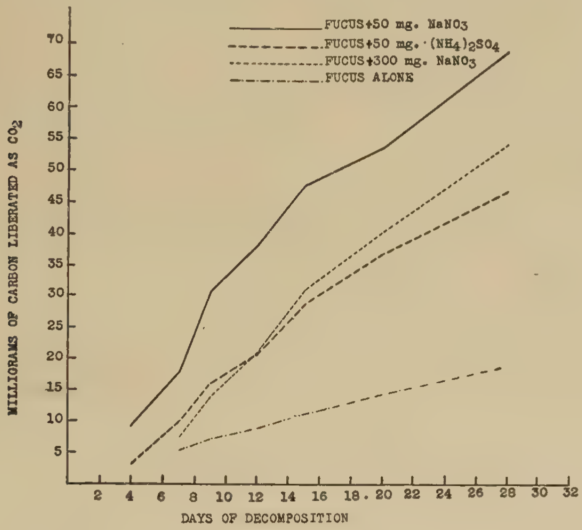

FIG. 4. Influence of available nitrogen upon the evolution of CO_2 in the decomposition of *Fucus vesiculosus* in sea water culture.

for the synthesis of bacterial cell substance. The cultures receiving an excess of nitrate showed a marked loss of the nitrate, through the process of denitrification. The fact that the nitrogen added to the cultures was required by the bacteria for the synthesis of cell substance can be further illustrated by the following considerations: in the decomposition of the algal material, the ratio between the carbon liberated as CO_2 and the nitrogen consumed by the bacteria, for the cultures receiving ammonium salt as a source of nitrogen, was $47.2:5.9 = 8:1$; the corresponding ratio for the cultures receiving the lower nitrate concentration was $8.7:1$. As will be shown later, these ratios are quite normal for the decomposition of carbohydrates by marine bacteria. There was no nitrate left in the cultures receiving the lower amount of nitrate, at the end of the decomposition period, and only a trace of nitrite was present; in the case of the higher nitrate concentration, only a small amount of nitrate was left at the end of the decomposition period, while a considerable amount of nitrite could be demonstrated in the medium.

The above results point definitely to the conclusion that nitrogen in the form of ammonia and nitrate can be used by marine bacteria for synthetic purposes, in the process of decomposition of nitrogen-poor algal material. When a high nitrate concentration is present, the *Fucus* material may be used as a source of energy by denitrifying bacteria for the reduction of the nitrate.

In order to determine what rôle the bacteria play in the decomposition of the *Fucus* material, 1-cc. portions of the various cultures were plated out, at the end of the decomposition period, using glucose-peptone sea water agar. The plates were incubated and the bacterial colonies counted at the end of 4 days. The results (Table V) show that there is a direct parallelism between the development of the bacteria and the liberation of CO_2, in the decomposition of *Fucus* material in sea water medium.

The results of these experiments prove conclusively that the rate of decomposition of marine algæ and the liberation of nitrogen as ammonia depend upon the chemical composition of the algal material. The more rapid decomposition of the *Ulva* than of the *Fucus* is due, aside from any difference in the chemical composition of the two, to the greater nitrogen content of the former. The more limited decomposition of the *Fucus* in the water cultures was due to a lack of available nitrogen, which prevented the development of the bacteria capable of decomposing the algal residues.

On comparing the results of Tables IV and V, one notes that there was a greater decomposition of the *Fucus* in the mud than in the sea water, even with the addition of an available source of nitrogen. This suggests the possibility that nitrogen is only one factor limiting the decomposition of marine algal material in the sea. Possibly the mud contained other elements which are also needed for bacterial growth and which were not present in sufficient amounts in the sea water. The greater decomposition of the *Fucus* material in the mud may also be due to the presence in the latter of specific bacteria, which are able to decompose certain organic constituents of the *Fucus,* while these bacteria are not present in the free water.

ISOLATION OF CELLULOSE AND HEMICELLULOSE-DECOMPOSING BACTERIA FROM THE SEA

In order to study further the mechanism of the decomposition of algal residues in the sea by bacteria, it was essential to isolate pure cultures of such bacteria and study their action upon certain purified algal constituents. The available information concerning the decomposition of specific constituents of marine plants by bacteria is largely limited to the agar-liquefying bacteria. Beginning with the work of Gran (4) in 1902, there were isolated from the sea a number of organisms capable of liquefying and decomposing agar. In most cases, as in the original form described by Gran, the agar was merely softened and, since no sugar was demonstrated, it is doubtful whether actual decomposition of the agar took place. Other organisms, as the form isolated from the marine mud of the Bahama banks and studied by Waksman and Bavendamm (18), decomposed the agar actively, liberating a large part of the carbon as CO_2; the bacteria, using the agar as a source of energy, were able to synthesize considerable cell substance.

Little is known concerning the occurrence in the sea of organisms capable of decomposing cellulose. Issatchenko (quoted in ref. 13) isolated from the mud of the salt lake Ssaky in Crimea two organisms, one of which decomposed cellulose without forming a pigment (*Bact. cellulosæ album*), while the other colored the paper yellow (*Bact. cellulosæ flavum*). Rubentschik (13) isolated from the salt limans in southern Russia an *Actinomyces* (*A. melanogenes*) capable of slow decomposition of paper. In 1929, Van der Lek (16) demonstrated the occurrence in the sea of *Bact. agar-liquefaciens,* which is capable of attacking cellulose and agar.

The following investigations deal with the occurrence in the sea of bacteria capable of attacking the various chemical constituents of marine residues as well as with the mechanism of the decomposition processes. The carbohydrate constituents were at first selected, due to their predominance in the algal residues. Three methods were employed for the isolation of the bacteria from sea water and from marine bottom material:

1. *The Silica-gel Method.*—Silica gel plates prepared by a method described elsewhere (19) are placed in flowing tap water, so as to bring about the removal of the excess of chlorides by dialysis; the dishes are then transferred to boiled sea water and kept there for about 1 hour; the last step may be repeated, using another portion of freshly boiled sea water, to insure sterility. The dishes are carefully removed from the water, the excess water drained off, and the surface covered with a small amount of powdered $CaCO_3$; for the purpose of isolating cellulose-decomposing bacteria, ground filter paper is placed on the surface of the gel; for the isolation of agar-decomposing organisms, powdered agar is used. A sterile salt solution consisting of 4 grams $NaNO_3$ and 2 grams K_2HPO_4 in 100 cc. of distilled water is then added to the surface of the medium, using 1-cc. portions for the small plates (15 cm. diameter) or 5-cc. portions for the large plates (25 cm. diameter). Plates so prepared were inoculated with fresh sea water, with marine bottom material or with pieces of algal growth.

2. *Enrichment Culture Method.*—This consists in placing 250-cc. portions of fresh sea water, 0.5 gram $NaNO_3$ and 0.1 gram K_2HPO_4, with or without 2-gram portions of $CaCO_3$, into a series of 250-cc. Erlenmeyer flasks. One per cent of cellulose, in the form of ground filter paper, or 1 per cent of dry agar were used as sources of carbohydrate.

3. *The Nutrient Agar Plate.*—This is made up to contain 1 gram of glucose, 1 gram peptone, 0.5 gram K_2HPO_4 and 15 grams agar in 1000 cc. of sea water. Marine mud, plankton tow, or fresh sea water are plated out on this medium, using various dilutions.

The cellulose-decomposing bacteria can be demonstrated most readily by the silica-gel plate method, while the agar-liquefying bacteria are best studied by the nutrient-agar plate method. In the case of the sea water, where only few bacteria are present, the enrichment culture may have to be used first, in order to demonstrate the presence of the specific organisms in the water. The cellulose bacteria developing on the silica gel plates are transferred to test tubes containing 5-cc. portions of sterile liquid medium (2 grams $NaNO_3$, 0.5 gram K_2HPO_4, 12 grams $CaCO_3$ in 1000 cc. sea water), with a strip of paper partly immersed in the liquid and partly above it. The agar-liquefying bacteria can be readily obtained in pure culture by the use of the agar plate; the colonies are picked and transferred to agar slants, and, if necessary, the process may be repeated using various dilutions of the culture. In the case of the cellulose bacteria, however, one must have recourse to the use of very high dilutions and repeated transfers in order to obtain pure cultures; this is true, of course, of the highly specific bacteria, which use cellulose as the only source of energy. When an organism is able to attack both cellulose and agar, its purification does not represent any particular difficulties.

The presence in sea water and in the sea bottom of bacteria capable of attacking cellulose could easily be established. These organisms were found to be particularly abundant in the plankton tow. Attention has already been called (20) to the fact that a definite parallelism was found to exist between the numbers of bacteria and diatoms in the sea water and in the plankton; the suggestion has been made that bacteria live in the free water only to a very limited extent, but their life and activities in the sea are closely associated with the development of the plankton organisms, in addition, of course, to the bacteria inhabiting the sea bottom.

A sample of diatom tow obtained from George's Bank (Station 1335) (20) gave an abundant growth of cellulose-decomposing bacteria. Similar results were obtained when the water and bottom mud from several stations in the Gulf of Maine and George's Bank were used for the inoculation of silica gel plates. It was thus possible to demonstrate the presence in the sea of a number of cellulose-decomposing bacteria, which represent a variety of types, as determined by their morphological, cultural, and biochemical characteristics.

Morphologically, the marine cellulose-decomposing bacteria were found to comprise short rods, spiral-forming organisms, vibrios, and undulating elongated rods with tapering ends, characteristic of the Cytophaga group. One is able to demonstrate without any difficulty the presence in the sea of the three groups of cellulose-decomposing bacteria which have been classified by Winogradsky (24), namely, *Cytophaga* (elongated, flexuous rods), *Cellvibrio* (bent rods with rounded ends), and *Cellfalcicula* (spindle and sickle-shaped). In crude culture, the cellulose-decomposing bacteria were invariably accompanied by numerous protozoa, including flagellates, ciliates and amœbæ, which feed upon the bacteria. All the bacteria were actively motile.

The marine cellulose-decomposing bacteria produce in culture a variety of pigments, namely, pink, orange, yellow, brownish-yellow, or no pigment at all. Most of them are aërobic. Some anaërobic forms were also demonstrated, which were found capable of producing gas actively with cellulose as the only source of energy. These organisms were not isolated, however, in pure culture and were only insufficiently studied. As to the utilization of carbohydrates, some of the cellulose-decomposing bacteria were found to be highly specific, being unable to use any other source of energy, except cellulose. Some attacked not only cellulose but also agar and other hemicelluloses, as will be shown later. Some were able to utilize a variety of sugars.

Agar-liquefying bacteria are even more commonly found in the sea; they are especially abundant in the diatom tow and on marine algæ and their residues, where they seem to find the necessary nutrients for their development. It was pointed out elsewhere that 1 cc. of diatom tow was found to contain 2,100 to 2,500 cells of bacteria capable of liquefying agar; they made up 5.7 to 6.7 per cent of the total number of colonies developing on the plate.

A number of bacteria were isolated, either directly from the plate or after preliminary enrichment culture with cellulose or agar in sea water. Crude cultures of cellulose-decomposing bacteria on the silica gel plate or in enrichment cultures of cellulose in sea water invariably yielded agar-liquefying bacteria. In some cases, these bacteria could decompose both cellulose and agar, while in other cases the agar-liquefying organisms merely accompanied the cellulose-decomposing forms. The bacteria thus isolated were found to belong to several groups varying considerably in their physiological characteristics. No attempt will be made to describe and classify these organisms. Three cultures were selected for a more detailed study of the decomposition of carbohydrates; the cultural and morphological characteristics of only these cultures are presented here.

Culture No. 2 (b) was isolated from the silica gel plate containing cellulose as the only source of energy and $NaNO_3$ as a source of nitrogen. In crude culture it attacked both cellulose and agar and produced gas as a result of the decomposition of the agar. When freshly isolated, it produced lilac colored to pinkish-brown colonies, on the surface of the agar.

Culture No. 3 was also isolated from the same plate as No. 2 (b). It was found to form, however, two types of colonies, one pinkish in color and one colorless. The pinkish culture was later found to be the same as No. 2 (b), and was designated as 2 (a); it also decomposed both cellulose and agar. The second culture could not utilize cellulose, but acted upon agar alone and was designated as No. 3.

Culture No. 15 formed a yellowish to dirty-brown color upon agar and in liquid culture. It did not attack cellulose, but could utilize agar as the exclusive source of energy.

The agar used for these studies was the Bacto-Difco agar. For later studies, specially washed agar was prepared in quantity by the Digestive Ferments Co.

A brief description of these three agar-liquefying bacteria is given here.

Bacterium sp. No. 2. Forms long, slender rods; often filamentous and curved in young cultures. A few motile rods are present. Occasionally there appear in these cultures a few extremely motile individuals, small in size, which seem to be identical with some of those found in Cultures 3 and 15. The organism reduces nitrate, decomposes cellulose, and does not liquefy gelatin.

Bacterium sp. No. 3. Forms very short rods, often coccus-like, with an equal proportion of slightly longer, slender rods scattered throughout the field. A few rods of the same length but much thicker appear with polar bodies. A few actively motile rods, often granular, sometimes having the appearance of two very short rods joined together, are also found. The latter appear more frequently among the non-motile forms. This organism liquefies gelatin, does not decompose cellulose, and does not reduce nitrate.

Bacterium sp. No. 15. Microscopically similar to No. 3, except that it contains more motile individuals and fewer coccus-like forms. Cultural characteristics are the same as those of No. 3.

DECOMPOSITION OF VARIOUS CARBOHYDRATES BY CELLULOSE-DECOMPOSING AND AGAR-LIQUEFYING BACTERIA

When agar or other constituents of marine algæ are decomposed by agar-liquefying bacteria, the carbohydrates are used by the bacteria chiefly as sources of energy, as well as for synthesis of bacterial cell substance. Due to the fact that agar and the other algal carbohydrates usually contain very little nitrogen, an additional source of available nitrogen, either in organic or in inorganic forms, has to be added to the cultures. This nitrogen is consumed by the bacteria and is transformed into organic nitrogenous compounds which are the constituents of the bacterial cell substance. This is clearly brought out in Table VI, where

TABLE VI

Decomposition of agar by three marine bacteria. Milligrams of dry material.

Culture	Agar left*	Agar decomposed	CO$_2$ evolved as carbon	Agar left, as sugar†	Agar utilized	NO$_3$−N left	NO$_3$−N consumed	Ratio of carbon liberated as CO$_2$ to nitrogen consumed
Control	498	0	0	245	0	26.4	—	
Bacterium sp. No. 2	167	331	91.6	82	163	14.0	12.4	7.4
Bacterium sp. No. 3	144	354	74.7	71	174	13.2	13.2	5.7
Bacterium sp. No. 15	384	114	49.4	187	58	18.8	7.6	6.5

* The total carbon content of the culture was determined and calculated as agar, allowing 44 per cent carbon in the dry, ash-free agar.

† Residual culture was hydrolyzed by boiling for 1 hour with 2 per cent HCl; the sugar formed on hydrolysis was used as measure of residual agar.

the results of the decomposition of agar and consumption of nitrogen by the three bacteria described above are presented. One hundred-cc. portions of sterile sea water medium containing 0.6 gram air-dry agar and 0.2 gram $NaNO_3$ were inoculated with the bacteria and the cultures incubated for 30 days; the CO_2 liberated in the process of agar decomposition was determined by passing CO_2-free air over the cultures and absorbing the CO_2 in standard $Ba(OH)_2$ solution. The actual amount of

TABLE VII

Influence of nitrogen source and agar concentration upon the decomposition of agar by marine bacteria

Concentration of agar in medium	Nitrogen source	Agar left, as sugar in hydrolyzed residue				Agar consumed, as hydrolyzed sugar		
		Control	Bacterium 2 (a)	Bacterium 2 (b)	Bacterium 3	Bacterium 2 (a)	Bacterium 2 (b)	Bacterium 3
per cent								
0.5	$(NH_4)_2HPO_4$	147	0	0	147	147	147	0
0.5	$(NH_4)_2SO_4$	147	127	142	147	20	5	0
1.0	$NaNO_3$	265	127	81	265	138	184	0
0.5	$NaNO_3$	136	0	0	42	136	136	94

TABLE VIII

Utilization of mono- and di-saccharides by agar-liquefying bacteria. $NaNO_3$ as source of nitrogen, and 1 per cent carbohydrate.

Nature of sugar	Glucose				Galactose			
Organism	Control	Bacterium 2(a)	Bacterium 2(b)	Bacterium 3	Control	Bacterium 2(a)	Bacterium 2(b)	Bacterium 3
Residual sugar	430	439	392	68	395	386	401	68
Sugar consumed	—	0	38	362	—	0	0	327
NO_3-N left	15.3	15.5	15.7	6.5	15.8	15.5	16.2	10.2
NO_3-N consumed	—	0	0	8.8	—	0	0	5.6

Nature of sugar	Mannose				Sucrose *			
Organism	Control	Bacterium 2(a)	Bacterium 2(b)	Bacterium 3	Control	Bacterium 2(a)	Bacterium 2(b)	Bacterium 3
Residual sugar	411	417	417	53	452	410	424	214
Sugar consumed	—	0	0	358	—	42	28	238
NO_3-N left	15.3	14.8	11.6	9.3	14.8	15.3	11.6	9.3
NO_3-N consumed	—	0.6	3.8	6.1	—	0	3.2	5.5

* Measured as invert sugar.

agar decomposed by the bacteria is greater than can be calculated from the residual carbon in the medium, since the latter includes not only the residual agar, but also certain decomposition products and the bacterial cell substance synthesized.

The nature of the nitrogen in the medium is of considerable influence in the amount of agar decomposed by different organisms, as shown in Table VII. One hundred-milligram portions of the nitrogen source were used in all the cultures. Both nitrate and ammonium salts can be utilized by the agar-liquefying bacteria as sources of nitrogen. However, in the case of the ammonium salt, the nature of the acid radical is of importance: the phosphate is as readily available to the bacteria as the nitrate; the sulfate was either not used at all or only to a very limited extent. The concentration of the agar in the medium is significant, since, in the case of one organism at least (*Bacterium* No. 3), agar was actively attacked when present in 0.5 per cent concentration but not in 1 per cent.

The ability of agar-decomposing bacteria to utilize various lower and higher carbohydrates is brought out in Tables VIII and IX. Only one of the three cultures of bacteria used in this study could assimilate all the three monosaccharides tested, namely *Bacterium* No. 3; this form does not attack cellulose and is a rather weak hemicellulose-decomposing form, as shown in Tables VII and IX. All the three organisms could utilize sucrose, the most active form being the one that could use all three monosaccharides. The two strains of the active cellulose and agar-decomposing bacterium (*Bacterium* No. 2) did not attack galactose or mannose.

As to their ability to decompose polysaccharides, the different organisms were again found to differ markedly from one another. The two strains of No. 2, the most active agar-decomposing organism, also decomposed cellulose actively, as well as starch and mannan; however, only one of the two strains (2*b*) could utilize galactan and inulin. The weak agar-liquefying organism (No. 3), which could attack monosaccharides readily, was also able to decompose most actively starch, mannan, and galactan.

The three agar-liquefying bacteria were thus found to show a very distinct specificity as regards their ability to decompose and utilize various carbohydrates. Culture No. 2 (a) attacked readily cellulose and agar, as well as starch and mannan, and sucrose only to a limited extent; it did not use glucose, galactose, and mannose, nor inulin and galactan. The other strains of this organism (No. 2b) decomposed agar and cellulose most actively, as well as inulin, starch, mannan, galactan, and were also able to make some use of glucose and sucrose. Culture No. 3 could not decompose cellulose; it acted upon agar only to a very limited extent and under certain conditions; it was able to use, however, monosaccharides and disaccharides readily, as well as starch, mannan, galactan, and inulin. These three organisms represent only a few of a large number of bacteria present in the sea and capable of attacking various complex and simple carbohydrates.

TABLE IX

Decomposition of polysaccharides by agar-liquefying bacteria. NaNo$_3$ as source of nitrogen.

Carbohydrate	Agar				Inulin *				Starch			
Organism	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3
Reducing sugar in medium, *mgm*...	0	0	0	0	0	0	77	0	0	0	327	0
Hydrolyzed carbohydrate, *mgm.* sugar..........	265	127	81	265	205	204	82	189	476	63	354	0
Carbohydrate consumed, as sugar, *mgm*..........	—	138	184	0	—	0	123	16	—	413	122	476
NO_3-N left, *mgm*.	15.3	10.7	9.3	11.8	—	14.8	10.2	14.3	15.8	4.9	11.1	7.8
NO_3-N consumed *mgm*..........	—	4.6	6.0	3.5	—	0.5	5.1	1.0	—	10.9	4.7	8.0

Carbohydrate	Mannan				Galactan				Cellulose ‡			
Organism	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3
Reducing sugar in medium, *mgm*...	0	0†	23	0	0	0	0.5	0	488	335	300	495
Hydrolyzed carbohydrate, *mgm.* sugar..........	367	14	38	0	174	182	131	114	459	270	247	455
Carbohydrate consumed, as *mgm.* sugar..........	—	353	329	367	—	0	43	60	—	155	190	0
NO_3-N left, *mgm*.	—	6.9	7.4	9.3	—	14.1	9.3	10.2				
NO_3-N consumed, *mgm*..........	—	8.4	7.9	6.0	—	1.2	6.0	5.1				

* 0.5 per cent used.

† In this culture practically all the mannan has disappeared, with no reducing sugar left in medium; the duplicate, however, had 255 mgm. reducing sugar left.

‡ These figures do not represent reducing sugar, but residual cellulose.

Summary

1. A study has been made of the proximate chemical composition of zoöplankton and of certain marine algæ, of their decomposition by marine bacteria, and of some of the bacteria capable of attacking certain carbohydrate constituents of marine plant life.

2. The zoöplankton was readily decomposed by bacteria in sea water and in marine mud media, with the result that more than a half of the total nitrogen present in the plankton was liberated as ammonia. Although the ammonia produced in the water and in the mud cultures was practically the same; a much greater amount of CO_2 was liberated as a result of the decomposition of the plankton in the mud than in the water medium.

3. It is suggested that the protein constituents of the zoöplankton decompose alike in the water and in the mud cultures; however, other chemical constituents of the plankton, free from nitrogen, such as certain fats, or low in nitrogen, such as the chitins, seem to undergo more rapid decomposition in the mud than in the water.

4. In the decomposition of algal material by marine bacteria, the chemical composition of the algæ is of prime importance. In the case of *Ulva lactuca,* a green alga containing about 2 per cent nitrogen on a dry, salt-free basis, active decomposition took place both in the sea water and in the marine mud cultures. In the case of the brown alga *Fucus vesiculosus,* however, containing less than 1 per cent total nitrogen, very little decomposition took place in the sea water medium, but the material decomposed very actively in the mud medium.

5. The addition of inorganic sources of nitrogen to the *Fucus* material in the sea water cultures resulted in a much more active decomposition, due to the fact that this nitrogen was required by the bacteria decomposing the *Fucus* constituents for the synthesis of their cell substance. The presence of *Fucus* material in sea water may, therefore, result in a minimum amount of available nitrogen. A definite parallelism was found between the development of the bacteria in the culture and the amount of decomposition of the *Fucus* material, as measured by CO_2 evolution.

6. Nitrate and ammonium salt were used readily as sources of nitrogen in the decomposition of the *Fucus* material by bacteria. The addition of an excess of nitrate to the culture proved to be injurious. This excess nitrate was rapidly destroyed by denitrifying bacteria.

7. The sea is found to harbor an extensive population of bacteria capable of utilizing cellulose and hemicelluloses. These bacteria represent a number of distinct types, varying considerably in their mor-

phological and physiological properties. Some of the bacteria were found capable of attacking both cellulose and agar, while others decomposed either cellulose alone or agar alone. Some of these bacteria are also able to decompose a number of other polysaccharides, as well as various mono- and disaccharides.

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Contribution No. 18

MARINE BACTERIA AND THEIR RÔLE IN THE CYCLE OF LIFE IN THE SEA

II. Bacteria Concerned in the Cycle of Nitrogen in the Sea[1]

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Introductory

Among the various elements which are essential for the growth of plant and animal life in the sea and which are present in minimum concentrations, nitrogen occupies a prominent place. Neither the plant nor the animal forms are able to use the gaseous nitrogen of the atmosphere, a function now known to be limited chiefly to certain specific groups of bacteria, and depend for their growth upon the combined nitrogen present in the sea; but even this form of nitrogen must be in a soluble, mineralized state before it can be assimilated by the plants. The plants transform the soluble forms of nitrogen into complex organic forms; upon the death of the plants, as well as of the animals which feed upon them, the nitrogen is brought again into circulation, through the action of a number of bacteria, in the form of ammonia. However, before this nitrogen is again assimilated by the marine plants, the ammonia is usually oxidized first to nitrite and then to nitrate by the action of certain other bacteria. The nitrate, if not consumed by the plants especially in the surface layers of the sea, may be reduced, under certain conditions, by the action of still other bacteria to nitrite, to ammonia, and to gaseous forms of nitrogen. Some bacteria may compete in the sea with higher plants for the available inorganic nitrogen, in the presence of certain carbohydrates, and thereby transform this nitrogen into organic compounds.

The bacteria are thus concerned in the sea with at least five distinct processes which are based upon the circulation of nitrogen, and which affect in numerous ways, directly and indirectly, the growth of plant and animal life in the sea, namely (1) liberation of nitrogen as ammonia, (2) oxidation of ammonia to nitrite and nitrate, (3) reduction of nitrate to nitrite and atmospheric nitrogen, (4) fixation of nitrogen, and (5) assimilation of inorganic forms of nitrogen. The first and last of these processes have been discussed at some length in a previous publication (48). The investigations reported in this paper are concerned with the other three processes of nitrogen transformation.

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Occurrence of Nitrifying Bacteria in the Sea

Historical

The formation of nitrate in the sea, as a result of oxidation of ammonia, is usually considered as the final step in the transformation of nitrogen, before it is made again available for assimilation by green plants; this does not exclude, of course, the probability that ammonia itself, the final nitrogenous product of decomposition of organic matter by bacteria, and nitrite, the first product of oxidation of ammonia, can also be utilized by marine algæ and by the chlorophyl-containing members of the plankton, as sources of nitrogen. Among the various marine problems, for which bacteria are believed to be responsible, none has aroused more discussion and greater interest than the process of nitrate formation in the sea, with the possible exception of nitrate-reduction by bacteria. This interest is due to the importance of nitrate formation in the metabolism of the sea and to the difficulty of studying the bacterial agents responsible for this process. An exact analogy is found in the study of the agents of nitrification on land: even forty years after the respective organisms have been isolated and cultivated by Winogradsky, papers still continue to appear which not only question the rôle of these organisms in the process but frequently doubt their very existence.

Boussingault looked upon the ocean, in 1860, as an immense reservoir of nitrogen in a combined form. Schlösing (43) demonstrated, in 1875, that while land waters are richer in nitrate, sea waters are richer in ammonia. It was recognized, however, that both ammonia and nitrate result from the decomposition of nitrogenous organic matter in the sea. Natterer (36) measured the NH_3, NO_2, and NO_3 content of sea water and found that the first was most abundant, while the last two were present in mere traces or were entirely absent; he believed that nitric acid produced by electric discharges sooner or later reaches the sea, enters there into organic combination, and is finally transformed to ammonia; this diffuses then into the atmosphere, and contributes again to the growth of plants on land; these results were not confirmed, however, by subsequent investigators. Brandt (9, 10) stated emphatically that the cycle of nitrogen in the sea is essentially not very different from that on land.

The first suggestion concerning the existence in the sea (Gulf of Naples) of bacteria responsible for the process of nitrification was made in 1898 by Vernon (45). Baur and Brandt (2) are believed to have demonstrated in 1900 the presence of nitrifying bacteria in the sea: two out of three mud samples inoculated into a solution containing ammonium salts gave active nitrification; however, sea water itself seemed to be free from the organisms concerned in this process. These results could not be confirmed by Gran (19) and Nathanson (37), who were unable to demonstrate these organisms either in the Norwegian fjords or in the Gulf of Naples. Gran found nitrifying bacteria only close to the shore. This led Nathanson to conclude that when bacteria are found in the sea not far from land, it is due to their introduction from the land soils by streams and land drainage. Gran and Nathanson adhered, therefore, to the earlier hypothesis of Schlösing that nitrates are not formed in the open sea, but are brought there from the outside, either from the atmosphere or from land.

Brandt argued that, if the nitrate comes into the sea either from the atmosphere or from land by means of streams and rivers, one would expect to find nitrate more abundant in the surface layers of the sea rather than in the lower depths, which is contrary to actual facts. The abundance of nitrate in the deeper layers of water led Brandt to conclude that nitrification takes place chiefly in the sea bottom or close to it. It was recognized, however, that close to the mouth of the rivers the relative concentration of nitrate was greater than in the open sea, but this was also found to hold true for other forms of nitrogen, namely ammonia and protein.

Thomsen (44) demonstrated the presence of nitrite-forming bacteria in considerable abundance in the sea bottom, although they were absent in the sea water and on algal material; the nitrate-forming organisms were also present in the bottom material, but only close to shore. The organisms responsible for the two processes, namely *Nitrosomonas* and *Nitrobacter,* were isolated from the sea and were found to be morphologically the same as the corresponding forms isolated by Winogradsky from land soil. The marine nitrite bacteria were considered as adaptation forms, their optimum temperature being similar to that of the bacteria from land. Thomsen found nitrifying bacteria not only in the mud from the Gulf of Naples, but also from the Kiel Bay and the North Sea. He believed that the negative results of

Nathanson were due to the nature of the medium which he had used for demonstrating the presence of these organisms, and to the fact that the cultures were not incubated sufficiently long and that the temperature was unfavorable. The occurrence of these bacteria on the bottom of the sea and not in the free water itself was explained by the fact that the water contains only traces of ammonia, while the continuous decomposition of plant and animal residues in or on the bottom supplies the necessary substrate for their action.

Issatchenko (24, 25) reported in 1908 that he found nitrifying bacteria in the water of the northern Arctic Sea; these organisms were present in the bottom material of the Catherine Coast (Murmansk) and of the North Ice Sea, as well as of the high seas; they were absent, however, in the surface water. Only nitrite-forming bacteria were found in the sea bottom, but not the nitrate-formers. In a later contribution (26), Issatchenko has shown that the nature of the sea bottom material is of importance in determining the abundance of nitrifying bacteria; these organisms were more abundant and could be more readily demonstrated in sandy bottoms and in shell-rich bottoms than in clay bottoms.

Liebert (30) attempted, in 1915, to isolate nitrifying bacteria from the water of high seas and from the ocean bottom; his results were entirely negative. He concluded that sea waters at a distance from land contain no bacteria capable of oxidizing ammonia and nitrate, due to the low content of these nitrogenous compounds in the sea. Marine mud from the North Sea also gave negative results, except in close proximity to shore. However, both nitrite and nitrate formation took place in the Zuyder Sea. Liebert believed that the FeS present so abundantly in the bottom of the ocean may play an important rôle in the oxidation of the ammonia in marine bottoms. Berkeley (14), in 1919, tested sea water for the presence of nitrifying bacteria with negative results, even after three months incubation of the cultures. As a result of a series of investigations, Lipman (31) concluded that although nitrifying bacteria are absent in the water of the open sea, they are present in the sea bottom, such as calcareous sand. Similar results were reported by Harvey (21–23).

On the basis of these results, Brandt (11) concluded in 1926, with much justification, that the results so far obtained are sufficient to establish definitely the fact that bacteria capable of oxidizing ammonium salts are completely lacking in surface waters, but are present in marine bottoms.

The possibility of photo-chemical oxidation in the sea of ammonia to nitrite and even to nitrate has recently been suggested (51). It has been known (39) that solutions of ammonia and ammonium salts exposed to sunlight, in the presence of small quantities of a photo-sensitizer, will give rise to nitrite, especially in alkaline solutions. TiO_2, ZnO, CdO, and others act as photo-sensitizers. The photochemical formation of nitrite and nitrate can, at best, however, explain only partly the origin of nitrates in the sea, since it must take place in the surface layers, with the formation of ammonia preceding this process. The fact that the nitrate is largely found in the lower layers of water would tend to emphasize the probable limitation of this process in the formation of nitrate in the sea.

Experimental

In an attempt to study the occurrence and activities of nitrifying bacteria in the sea, it was deemed essential to establish at first the conditions favorable for the growth of these organisms. One deals here with bacteria highly selective in their metabolism, very sensitive to environmental conditions, and specific in their food requirements, as was amply shown for the corresponding organisms universally active and abundant in land soils. Whether or not the bacteria responsible for nitrite and nitrate formation in the sea are as highly sensitive to conditions as the land bacteria, the first prerequisite in such investigations was to select a medium favorable for their development under artificial laboratory conditions. Several preliminary experiments were, therefore, carried out in order to test sea water as a medium and the most optimum conditions for the growth of these organisms.

At first, fresh sea water, to which 0.05 per cent K_2HPO_4, some $CaCO_3$, and varying amounts of ammonium salts had been added, was used. These experiments have shown that neither nitrate nor nitrite was formed in such a medium. However, when fresh sand or mud was introduced into the flasks, active nitrite formation took place. The results of a typical experiment are reported in Table I. The medium used in this experiment consisted of,

Fresh sea water	1000 cc.
K_2HPO_4	1 gram
$(NH_4)_2SO_4$	1 gram
$CaCO_3$	5–10 grams

This medium was placed in a series of flasks; these were plugged with cotton and the medium left unsterilized. Some of the flasks received 30-gram portions of fresh bottom mud taken off the shore of Gay Head, while others did not receive any mud. At frequent intervals nitrite tests were made by removing 1-cc. portions of the culture, by the use of sterile pipettes. The test was made with a mixture of sulphanilic acid and diethyl β-naphthylamine solutions. One cc. of culture was diluted with 5 cc. of distilled water and 1-cc. portions of each of the two reagents added. The color was read after 5 or 10 minutes.

The results brought out in Table I clearly demonstrate the fact that sea water either does not contain any bacteria capable of oxidizing ammonium salts to nitrite or is not a favorable medium for the development of these bacteria. In the culture to which marine mud has been added active nitrite formation took place, even after 20 days incubation; the amount of nitrite formed increased rapidly on further incubation. This can be due either to the presence of nitrifying bacteria in the mud or to the fact that the mud made the sea water medium more favorable for the development of these organisms.

TABLE I

Nitrite formation in sea water medium and in sea water medium to which fresh marine mud has been added.

Tr = trace of nitrite; 0 indicates negative test; + = positive nitrite test; +++ = extensive nitrite formation; ++++ = maximum nitrite reaction; – indicates culture discarded.

Treatment of culture	Days of incubation of cultures						
	6	15	20	24	28	33	38
Water alone	0	0	0	0	tr	tr	tr
Water alone	0	0	0	0	tr	–	–
Water and mud	0	0	+	+++	++++	++++	++++
Water and mud	0	0	+	+++	++++	–	–

Another important point to be noted from this experiment is that only the tests for nitrite were positive, while the tests for nitrate were all negative, even in the mud cultures. Attention has been called, in the review of the literature, to the comparative rarity in the sea of the nitrate-forming bacteria, as compared with the nitrite-formers. This would seem to be an anomaly, since there is very little nitrite present in the sea as compared with nitrate. If one remembers, however, the fact that the nitrate bacteria are highly sensitive to traces of free ammonia, which would be produced from the ammonium salt in an alkaline medium, one would expect to find these organisms, if they are present at all, only after all the ammonia has been oxidized to nitrite. The tests were continued for a longer period, but no trace of nitrate could be detected within 57 days of incubation; however, after 60 to 62 days, the mud containing cultures gave a definite test for nitrate, and after 77 to 84 days, the test became strongly positive.

An experiment was then started to determine the influence of the initial concentration of ammonium salt added to the original medium upon the development of nitrite and nitrate-forming bacteria, since some of the previous investigators (31, 33) considered this to be an important factor. This experiment was carried out as follows: Twelve 250-cc. Erlenmeyer flasks received 40-gram portions of washed sea sand and 2 grams $CaCO_3$; the flasks were plugged with cotton and sterilized for 1 hour under pressure. Sixty-cc. portions of sea water containing 0.1 per cent K_2HPO_4, previously heated for 1 hour at 70° C., were then added to the flasks, as well as varying amounts of an ammonium sulfate solution, also heated previously at 70° C. Five of the flasks were inoculated with fresh surface water taken from Great Harbor, near the Oceanographic Institution wharf, five flasks were inoculated with sea bottom mud from off the shore of Gay Head and 2 flasks with 4 drops of an enriched culture of nitrite-forming bacteria grown in a sand-sea water medium.

TABLE II

Effect of varying concentrations of ammonium sulfate upon nitrite formation in sea water-sand medium.

0 indicates negative test; + = positive nitrite test; ++ and +++ = extensive nitrite formation; ++++ = maximum nitrite formation.

Concentration of $(NH_4)_2SO_4$ in 60 cc. of medium	Sea water inoculum					Mud inoculum					Enriched culture inoculum			
	Days of incubation					Days of incubation					Days of incubation			
	3	7	12	17	20	3	7	12	17	20	3	7	12	17
mgm.														
0	0	0	0	0	0	0	0	+	++++	++++				
10	0	0	0	0	0	0	0	++	++++	++++				
25	0	0	0	+	+++	0	0	0	+	++	0	0	+	++++
50	0	0	0	+	++	0	0	+	+++	++++	0	0	+	++++
100	0	0	0	0	0	0	0	0	++	++++				

The concentration of the ammonium salt and the nature of the inoculum were found to have an important effect upon the rapidity of nitrite formation, as shown in Table II. The use of sea water as an inoculum gave no nitrite formation with either the highest or the lowest concentrations of the ammonium salt; however, a positive reaction was obtained in the cultures containing 25 and 50 mgm. of the salt, after 17 days incubation. The cultures inoculated with mud gave a positive nitrite test in some cultures in 12 days and in all cultures in 17 days. The mud cultures behaved in a manner similar to the enriched culture of the nitrite-forming organism.

Some of the cultures in the above experiment were incubated for a longer period than that reported in Table II. The culture containing 10 milligrams of ammonium sulfate and inoculated with mud gave very abundant nitrite formation up to 34 days; after 42 days, however, the nitrite disappeared. It had been completely and rapidly converted to nitrate.

In the previous experiments, both the water and the marine bottom material were obtained in the proximity of land. The following experiments deal with the occurrence of nitrifying bacteria in the Gulf of Maine and on George's Bank, at a considerable distance from shore. Material for this experiment was obtained on a cruise of the "Atlantis," which took place during August 1–5, 1932, and which has been described in detail elsewhere (50). For this purpose a series of flasks were prepared containing the following materials:

TABLE III

Presence of nitrite-forming bacteria in the sea water of the Gulf of Maine (Stations 1329, 1330, 1331, and 1332) and Georges Bank (Stations 1333 and 1334) at different depths.

Tr = trace; 0 indicates negative test; + indicates positive reaction; ++ = extensive nitrite formation; +? indicates doubtful reaction. For map showing location of stations, see Rakestraw, 1933, *Biol. Bull.*, **64**: 150.

Station No.	Depth of water	Days of incubation			
		10	15	20	23
	meters				
1329	Surface water	0	+?	0	0
1329	Above bottom	0	0	0	0
1330	Above bottom	0	0	0	0
1331	Surface water	0	0	0	0
1331	30	tr	tr	tr	+
1331	50	tr	+	+	+
1331	100	tr	0	0	+
1331	215	tr	+	+	+
1331	Above bottom	0	0	0	0
1332	Surface water	0	+	+	++
1332	50	0	+	+	+
1332	100	0	+	+	+
1333	Surface water	0	tr	tr	tr
1334	Surface water	0	tr	0	tr
1334	Deep water	0	tr	tr	tr

Sand	15	grams
$CaCO_3$	1	gram
K_2HPO_4	0.005	gram
$(NH_4)_2SO_4$	0.01	gram

The flasks were plugged with cotton and sterilized, at 15 lbs. pressure, for 15 minutes. A standard solution of ammonium sulfate was sterilized separately and added to the sterile flasks. At the various stations visited about 50–60-cc. portions of fresh sea water, brought up from the different depths, by means of sterile glass containers, were added to the flasks immediately after the samples were obtained. To determine the occurrence of nitrifying bacteria in the marine bottom of the open sea, a medium similar to the above and containing 75–cc. portions of sea water with only 5 milligrams of $(NH_4)_2SO_4$ per flask was prepared. This medium was sterilized for 1 hour in flowing steam (100° C.). The flasks were inoculated, after returning from the cruise, with small quantities (about 1 gram) of fresh mud, obtained in the Gulf of Maine under sterile conditions; this was done by removing carefully the inner part of a core of mud.

Table IV

Occurrence of nitrite-forming bacteria in the marine bottom of the Gulf of Maine (Stations 1329 and 1330) and Georges Bank (Station 1336).

Tr = trace of nitrite; + = positive nitrite test; ++ and +++ = extensive nitrite formation. For map showing location of stations see Rakestraw, 1933, *Biol. Bull.*, **64**: 150.

Station No.	Depth of mud	Days of incubation			
		2	6	11	15
	cm.				
1329	0–30	+	++	+++	+++
1329	30–60	0	++	++	+++
1329	60–90	0	tr	tr	tr
1330	0–30	+	+++	++	+++
1330	30–60	0	tr	tr	tr
1330	60–90	tr	tr	tr	tr
1336	Surface layer of sand bottom	0	+	+	++
Control	0	0	0	0	

The results of these experiments on the presence of nitrite-forming bacteria in the sea water and in the marine bottom of high seas, are reported in Tables III and IV. These results show that the free water in the high seas is either entirely free from nitrifying bacteria or contains only very few cells of these organisms. In the case of the marine bottoms, however, positive nitrite formation was obtained in the case of the mud bottoms (Stations 1329 and 1330). Even within two days incubation these bacteria seem to be present in the mud to quite considerable depths. The sand bottom also gave active nitrite formation, somewhat more slowly than the mud bottoms.

In addition to the cultures containing ammonium salts, other cultures containing nitrites were prepared, but all attempts to obtain nitrate-forming bacteria in these media failed. However, when old cultures of nitrite-forming bacteria, in which nitrate-formation began to take place, were transferred to fresh media containing 5 mgm. KNO_2 in 30 cc. of medium, active nitrate formation took place within 7–8 days.

The following medium was finally adopted for the purpose of demonstrating the presence of nitrifying bacteria, using large test tubes instead of flasks, due to the ease of handling. The tubes contained 30 cc. sea water, 10 gm. washed sand, 1 gm. $CaCO_3$, 5 mgm. $(NH_4)_2SO_4$, and 5 mgm. K_2HPO_4. The medium was sterilized in flowing steam; the ammonium salt was sterilized separately, then added in aliquot portions, using sterile pipettes.

The nitrite-forming organism could be readily cultivated on the above medium; on repeated transfer, active cultures were obtained. By inoculating a silica-gel medium (thoroughly dialyzed in tap water and soaked in sterile sea water), to which an ammonium salt and calcium carbonate had been added, with an active liquid culture, abundant formation of nitrite on the plate took place within 14 days. This should facilitate greatly the isolation of the organism in pure culture, although for the purpose of the above experiments this was not considered essential.

The results presented here and other data of a similar nature are quite sufficient to demonstrate definitely that free sea water, especially at the surface of the sea, has either no nitrifying bacteria at all or only very few of these organisms. On the other hand, the sea bottom, mud or sand, has an active population of nitrifying organisms. The formation and accumulation of nitrate in the sea is probably due largely to the activities of these organisms. The processes of nitrite and nitrate formation take place in the sea bottom; the nitrate then diffuses into the water. The fact that it is much easier to demonstrate in culture the formation of nitrite than that of nitrate is due largely to the specificity of the organisms and conditions of cultivation.

Reduction of Nitrates in the Sea by Bacteria

Historical

Nitrate is consumed in the sea not only by the green plants but also by bacteria, which either transform it into bacterial cell substance or reduce it to nitrite, ammonia, or atmospheric nitrogen. The activities of the last group of bacteria have aroused the interest of oceanographers. The increasing concentration of nitrate with the depth of the water in the open sea, the high nitrate content in temperate waters and its comparative poverty in tropical waters, the fact that nitrate is the final product of the transformation of nitrogen by bacteria, and further the fact that the phytoplankton and marine algæ seem to use this form of nitrogen in preference to any other, have all served to center the attention of marine investigators upon the transformation of nitrate in the sea. An attempt has actually been made to correlate not only the relative abundance of nitrate in the sea but also the abundance of plant and animal life with the activities of the nitrate-destroying bacteria.

If one is to admit the assumption that bacteria control largely the cycle of nitrate in the sea, especially its disappearance under certain conditions and not under others, one would have difficulty in explaining the common observation that nitrate and phosphate occur in the sea in a parallel manner. If one were to conclude that the nitrate balance in the sea is controlled by the activities of nitrate-forming and nitrate-reducing bacteria, one must also postulate that the formation and disappearance of phosphate in the sea are also controlled by the activities of comparable groups of bacteria. This may be true for the liberation of nitrate and phosphate, since nitrate formation is a resultant of a chain of processes brought about by a series of bacteria, while phosphate is liberated directly, in the decomposition process of plant and animal residues in the sea. On the other hand, although nitrate-reduction is known to take place under favorable environmental conditions, which include the presence of nitrate, an available source of energy, and the necessary bacteria, the reduction of phosphate in the sea, aside from its direct consumption by the phytoplankton, is still a matter of conjecture.

It is further essential to keep in mind the fact that the nitrate-reducing bacteria do not comprise a single group of organisms but several groups with distinct physiological properties, namely, (*a*) bacteria which reduce nitrate to nitrite only, (*b*) bacteria which reduce nitrate and nitrite to ammonia, and (*c*) bacteria which reduce nitrate and nitrite to atmospheric nitrogen and to gaseous oxides of nitrogen. Only the last group should be considered as true denitrifying bacteria; their activities may be of significance in the disappearance of nitrate in the sea. The products formed by the first two groups of nitrate-reducing bacteria can either be assimilated directly by the marine algæ and the phytoplankton or they can be oxidized back to nitrate by specific bacteria.

According to Audoynaud (1), both ammonium salts and nitrates are assimilated by the numerous small organisms inhabiting the sea, their bodies later sinking to the bottom and forming layers of various degrees of thickness. He, as well as Schlösing (43), had only a very vague idea concerning the return of the elements, especially of the nitrogen, to the atmosphere or to the sea, as a result of the decomposition of the organic residues.

The presence in the sea of bacteria capable of reducing nitrate, largely to nitrite, was demonstrated by Beijerinck (5) in 1890, by Fischer (15) in 1894, for phosphorescent bacteria, by Russell (42) in 1893, for the sea in the vicinity of Woods Hole, and by Vernon (45) in 1898, for the waters in the Gulf of Naples.

A decided stimulus to further investigations of nitrate-reducing bacteria was given by Brandt (9), in 1899, by his now famous hypothesis concerning the function of denitrifying bacteria in controlling the supply of available nitrogen in the ocean and, therefore, of plankton development. Brandt believed that most of the nitrogen compounds are brought into the sea from land by the rivers, streams, and drainage waters; without the activities of the denitrifying bacteria, nitrate concentration in the sea would soon reach a point where it would become injurious to living organisms. The more abundant plankton life in shallow waters, as compared with deep waters, was ascribed to the fact that, in the former, the available inorganic nutrients are distributed through a smaller volume of water, while in the deeper seas, the nutrients are distributed in much greater volumes of water. Plankton-rich seas were found to contain much more nitrate (and nitrite) than plankton-poor seas; a direct relation was thus shown to exist between the mass of plankton development and the amount of nitrate present. The conclusion was, therefore, reached that the abundance of nitrate controls the abundance of plankton. The tropical and sub-tropical seas are known to be comparatively poor in plankton, while the arctic seas are rich in plankton, especially in the summer; this phenomenon is just the opposite of that found in the case of land vegetation. This difference in the plankton development in the sea was explained by the difference in the concentration of available nitrogen compounds; the tropical climate favors active denitrification which results in the reduction of nitrate and the limitation of this most important plant nutrient in the sea.

These ideas resulted in an extensive study of the occurrence of denitrifying bacteria in the sea and their importance in marine processes. In 1902, Baur (2) reported the isolation of a denitrifying bacterium from the mud of an aquarium rich in nitrate; another form was isolated from the mud in the bay of Kiel. Gran (18) isolated from the sea, in 1901, several bacteria capable of reducing nitrate. He found that only at close proximity to shore, where organic residues are present in abundance and where large quantities of nitrate and nitrite are brought in from shore, is there any possibility that true denitrifying bacteria play an important part in the destruction of nitrate. Gran concluded that Brandt's hypothesis is untenable.

Feitel (14) has shown in 1903 that denitrifying bacteria are present in great abundance in the sea, at all depths, but more often on the sea bottom or in the water near it, where organic matter is abundant. These bacteria were found not only close to land but also at a considerable distance from shore; when isolated in pure culture, they were capable of bringing about active denitrification, especially at low temperatures. Nathanson (37) demonstrated in 1906 the presence of denitrifying bacteria in the Gulf of Naples. In 1908, Kühl (29) found that marine mud brings about active denitrification. In 1909, Gräf (17) isolated from the waters of the Atlantic and Indian oceans 17 forms of denitrifying bacteria; this common occurrence of nitrate-reducing bacteria, taken together with the observations of the increase in nitrate concentration with an increase in the depth of water and the decrease of nitrate concentration with an increase in temperature, was believed to substantiate Brandt's hypothesis.

Issatchenko (25) found denitrifying bacteria in the North Polar Sea, capable of growing at very low temperatures (1–3° C.). He concluded that the bacteria reducing nitrate to nitrite or to ammonia are widely distributed in the sea at various depths and are represented there by numerous types, which are normal inhabitants of the sea. The true denitrifying bacteria, which are also widely distributed in the northern seas, were considered of doubtful importance in bringing about any appreciable losses of nitrogen in the sea. The fact that these bacteria are able to develop and reduce nitrate at low temperatures (12) and the fact that warm seas are also rich in nitrates (16) led Issatchenko to deny the validity of Brandt's hypothesis.

According to Gran (20), appreciable denitrification may take place only in enclosed bays and fjords, where large amounts of organic matter are present, considerable nitrate is brought in from land, and, due to limited circulation, the amount of oxygen is limited.

This brief summary of the literature bearing upon Brandt's hypothesis, concerning the importance of denitrifying bacteria in the cycle of life in the sea, points to the fact that the weight of the evidence bears out the conclusions of Gran and Issatchenko, namely, that admitting the existence of denitrifying bacteria in the sea, their rôle in marine processes is far from being established; certainly their part in controlling the nitrogen content of the sea and, therefore, of plankton development is shown to be of minor importance.

Another hypothesis based upon the activities of denitrifying bacteria served to focus the attention of oceanographers on the rôle of these organisms in marine processes. Drew (13) attempted to correlate the activities of these bacteria with the process of calcium carbonate precipitation in the sea: nitrate-reducing bacteria decompose calcium salts of organic acids present in the sea; this results in the precipitation of the calcium as calcium carbonate. However, further studies on the occurrence of denitrifying bacteria in tropical seas and on the mechanism of calcium carbonate precipitation did not tend to confirm this hypothesis. Lipman (32) found that precipitation of $CaCO_3$ will take place in a suitable medium even in the absence of nitrate. Whatever the final explanation of the mechanism of calcium precipitation in the sea (32, 34, 3, 4), one is justified in concluding that the original hypothesis of Drew has not been substantiated by subsequent investigators. In order to bring about such extensive precipitation, large quantities of nitrate would have to be formed and still larger quantities of organic acids produced in the sea, two processes which have not been sufficiently established.

However, even if these two hypotheses concerning the importance of denitrifying bacteria in marine processes may be considered, for the present at least, as untenable, the fact remains that the sea harbors many bacteria capable of reducing nitrate to nitrite, to ammonia, and to atmospheric nitrogen (38, 35). Only a detailed study of the occurrence of these organisms in the sea and of their biochemical activities may enable one to interpret their true function in marine processes.

Experimental

Most of the media used by previous investigators for the growth of nitrate-reducing bacteria contained peptone (3, 34), which could serve both as a source of energy and of nitrogen. However, the use of an organic nitrogen compound for the growth of these bacteria tends to confuse the results obtained, since some of the products, such as ammonia, may either be due to the reduction of the nitrate or to the decomposition of the protein. In the following experiments, a synthetic medium was employed, with nitrate as the only source of nitrogen and with various carbohydrates or salts of organic acids as sources of energy.

In the first experiment, a study has been made of the relative abundance of nitrate-reducing bacteria in sea water and in marine mud. For this purpose the following medium was found to give good results:

Sea water	1000	cc.
Ca-acetate	10	grams
$NaNO_3$	1.0	gram
K_2HPO_4	0.5	gram
$FeSO_4 \cdot 7H_2O$	0.01	gram

Thirty-five-cc. portions of this medium and 10-gram portions of sand were placed in a series of large test tubes; these were plugged with cotton and sterilized, by heating at 70° C. for 4 hours. By the use of this medium and the dilution method, denitrifying bacteria, or organisms capable of reducing nitrate to gaseous forms of nitrogen, were found in dilutions of 1 cc. and 0.1 cc. of sea water, taken near shore, and in 0.0001 gram of mud.

The above method of sterilization proved to be unsatisfactory, since some of the controls contained bacterial spores. A study has, therefore, been made of the influence of sterilization of the medium upon the growth of nitrate-reducing bacteria. The results of this experiment are presented in Table V. Autoclaving the medium did not render it unfit for the development of these bacteria, hence one could feel fully justified in proceeding with this method of sterilization.

The above medium was used in the investigation of the distribution of nitrate-reducing bacteria in sea water and in mud in the Gulf of Maine. The results of this experiment, reported elsewhere (50), brought out the fact that bacteria reducing nitrate to nitrite are universally distributed in the sea. However, the occurrence in the open sea of bacteria reducing nitrate to atmospheric nitrogen is very limited. Only in the case of one station, with 0.1 gram of mud as the inoculum, was positive denitrification observed, when the nitrate was rapidly reduced to gaseous nitrogen. In none of the other seven stations studied and in none of the water samples obtained from the various depths could the presence of these organisms be demonstrated. The bacteria reducing nitrate to nitrite were found to be much more abundant in the mud than in the free water. Most of the nitrate-reducing organisms present in the water belonged to the non-spore-forming bacteria; how-

TABLE V

Influence of sterilization of medium upon the development of denitrifying bacteria. Marine mud used for inoculation and cultures incubated for 3 days.

Dilution of mud	70° C., 2 hours*	100° C., 1 hour	15 lbs. pressure, 15 minutes	Filtration of culture through Seitz filter*
0.01	Gas	Gas	Gas	Gas
0.001	Gas	Gas	Gas	0
0.0001	Pellicle, no gas	Pellicle and gas	Gas	0
0.00001	0	Pellicle, no gas	Medium turbid, surface ring	0
Control	0	0	0	0

* Tubes and sand sterilized under pressure.

ever, several tubes inoculated with various dilutions of mud showed unmistakably the presence of spore-forming organisms. The actual amount of nitrate reduced in all the cultures reducing nitrate to nitrite was comparatively small.

In order to study the process of reduction of nitrate under controlled laboratory conditions, especially for the purpose of determining the influence of the energy source upon the reduction of nitrate to nitrite and to atmospheric nitrogen, the following experiment was carried out: 50-cc. portions of fresh sea water containing 50 mgm. of $NaNO_3$ were placed in a series of large tubes and 25-gram portions of sand added. Small portions of dry algal material, using *Fucus vesiculosus* or *Ulva lactuca,* were added to some of the tubes; some cultures, with and without nitrate, were used as controls. Some of the tubes were left without further inoculation, while some were inoculated with marine mud. All the cultures were incubated at room temperature and readings taken at the end of 5 and 9 days. The water used in this experiment was taken near shore at the Woods Hole harbor, while the mud was obtained off the shore of Gay Head.

The results presented in Table VI show that algal material can be used readily as a source of energy for the reduction of nitrate by the bacteria living in the sea water and in marine mud. There were found several marked differences, however, between the different cultures, depending on the nature of the algal material and on the kind of inoculum. In the sea water medium, the organic constituents of the *Ulva* were used readily as sources of energy for the reduction of the nitrate, as shown by the rapid reduction of the nitrate to nitrite and to gaseous nitrogen within 5 days; after 9 days incubation, all the nitrate and nitrite have completely disappeared, due to their reduction to atmospheric nitrogen. When *Fucus* was added to the sea water, the reduction of the nitrate was much slower, no gas being formed and considerable nitrate and nitrite being still left after 9 days incubation. The sea water medium containing nitrate only and no additional energy source showed no trace of reduction of nitrate, either to nitrite or to gaseous nitrogen.

The cultures heavily inoculated with mud gave a totally different type of nitrate reduction. When the *Ulva* material was added to the medium, both the nitrate and nitrite disappeared in 9 days, with active gas evolution, even more rapidly than in the cultures of sea water alone. In the case of the cultures receiving the *Fucus* the reduction of the nitrate was so rapid that, within 5 days, all the nitrate and nitrite disappeared completely from the culture, accompanied by abundant gas evolution. The difference in the influence of the *Fucus* material in the cultures inoculated with water and with mud is due largely to the fact that, as suggested previously, marine mud probably contains bacteria capable of attacking the specific carbohydrates of the *Fucus,* while such bacteria are absent in the sea water itself. The mud-inoculated cultures receiving no algal material also gave active reduction of nitrate to nitrite and even to atmospheric nitrogen; in this case the small amount of available organic matter in the mud (46) was probably used

TABLE VI

Utilization of marine algæ as sources of energy for bacteria reducing nitrate.
Tr = trace of nitrite or nitrate; 0 indicates negative test; + = positive nitrite or nitrate test; ++ and +++ = considerable nitrite or nitrate present; +++++ = maximum nitrite formation.

Source of algal material	Mud inoculum	Growth of bacteria	Gas		Nitrite		Nitrate	
			5 days	9 days	5 days	9 days	5 days	9 days
Sea water alone.......		0	0	0	0	0	0	0
Sea water and nitrate......		0	0	0	0	0	++	++
Sea water, nitrate, and Ulva.......		Turbidity in culture	+	0	+++++	0	++	tr
Sea water, nitrate, and Fucus......		Turbidity and pellicle	0	0	+++	+++++	++	+
Sea water alone	+	0	0	0	0	+	0	0
Sea water and nitrate......	+	Turbidity	+	0	++	++	++	++
Sea water, nitrate, and Ulva.......	+	Turbidity	+	+	+	0	+	0
Sea water, nitrate, and Fucus......	+	Pellicle	+	+	0	0	0	0

as a source of energy. The traces of nitrite found in the culture which had not received any nitrate but which was inoculated with mud are possibly due to the formation of nitrite by the oxidation of the ammonia which is formed in the decomposition of the organic nitrogenous complexes in the mud.

A study of the results of the two experiments, namely, (1) those of the cruise of the "Atlantis," which pointed to the presence in the sea of bacteria capable of reducing nitrate chiefly to nitrite, and (2) those emphasizing the importance of the specific energy source for the activities of the nitrate-reducing bacteria, will justify one in drawing certain definite conclusions. The marine algæ and members of the phytoplankton live and synthesize their cell substance in the surface layers of the water, or in the zone of maximum photosynthetic activities. As a result of these synthetic processes, the plants consume the nitrate present in the particular layers of water in the sea. The bacteria present in the water or attached to the algæ or the phytoplankton feed upon certain secretion products of these marine plants or upon the plant detritus, after the plants die. Since these algal and diatom substances are either free from nitrogen, as in the case of some of the secretion products, or contain only very limited amounts of this element, the bacteria decomposing them, unless they are nitrogen-fixing organisms, have to draw upon the available nitrogen present in the surrounding medium, namely in the sea. This results in the consumption of a certain amount of nitrate by the bacteria. As a result of these two processes, namely phytoplankton development and bacterial consumption of the algal products, the nitrate tends to disappear in the surface layers of water in the sea.

Among the bacteria attacking the algal and diatom products and residues in the surface layers of the water are present organisms which not only consume the nitrate and transform it into organic nitrogenous compounds, as constituents of the bacterial cell substance, but which also reduce the nitrate. Such reduction will take place only in the presence of an excess of nitrate, above that consumed by the algæ and diatoms and the heterotrophic marine bacteria. This reduction is carried largely to the nitrite stage, but not to atmospheric nitrogen. One would, therefore, expect that where active plant growth takes place in the sea nitrites should be present rather than nitrates. This does not indicate any loss of nitrogen from the sea but merely that at a certain level below the surface of the water, conditions are favorable to the reduction of the nitrate to nitrite. The latter can, of course, be used by the growing algæ and diatoms as a source of nitrogen just as well as the nitrate itself.

The marine bottom presents a totally different bacteriological system from that of the water itself. Not only does it harbor an extensive bacterial population capable of reducing nitrate to nitrite but it may also contain true denitrifying bacteria. However, the occurrence of such bacteria in the mud does not at all indicate that the process of complete reduction of nitrate to atmospheric nitrogen is very pronounced even at the sea bottom. Here also, certain controls prevent the rapid losses of nitrogen in a combined form which is so essential for the life of the plants in the sea. In order to reduce the nitrate (or nitrite) to atmospheric nitrogen, the denitrifying bacteria must have a source of available energy; but the organic matter of the sea bottom, or the marine humus, can be used only to a limited extent as this source of energy. This marine humus contains carbon and nitrogen in such proportions (46) that its decomposition will give rise to available nitrogen in the form of ammonia, which is used as a substrate for the bacteria producing nitrate, through the nitrite stage. The only existing possibility for denitrification to take place is in the very surface layer of the marine bottom where incompletely decomposed plant residues may offer a source of available energy for the denitrifying bacteria. It is doubtful whether even under these conditions the nitrate-consuming bacteria, decomposing these residues, are not more predominant and more active than the nitrate-destroying bacteria.

Occurrence of Nitrogen-Fixing Bacteria in the Sea

Historical

Although the ocean is usually considered to be a large reservoir of combined nitrogen, the question as to the origin of this nitrogen has frequently been raised. Some investigators believed that it is a direct result of electric discharges, while others looked to the land as the sole source of this nitrogen; however, the belief expressed by some that bacteria are responsible for the presence of combined nitrogen in the sea has been gaining ground. There is no doubt that all these three theories have some justification. The presence of a considerable amount of nitrogen in marine mud (46), especially not far from land, points to the latter as a possible source of at least some of the nitrogen present in the sea; whether this nitrogen is readily available for the nutrition of marine algæ and plankton is another question, and one which is still little understood.

The function of the nitrogen-fixing bacteria in increasing the supply of combined nitrogen in the sea is difficult to determine, the results obtained under laboratory conditions not lending themselves readily to a direct application to natural marine processes. While the importance of the bacteria concerned in the fixation of atmospheric nitrogen on land is well established, the available information about the occurrence and activities of these bacteria in the sea is still very elementary. When one considers further the fact that, of the two groups of nitrogen-fixing bacteria on land, the function of only the symbiotic organisms, namely root-nodule bacteria, is well established, while the importance of the non-symbiotic bacteria is still in dispute, and that it is the latter group which is found in the sea, one can readily understand the reason for the lack of sufficient knowledge concerning the activities of these bacteria in the sea.

Benecke and Keutner (7) were the first to demonstrate that both the ærobic *Azotobacter* and the anærobic *Clostridium pastorianum* are found in the sea, the former occurring largely on the plankton, while the latter is present chiefly in the lower layers of water and in the sea bottom. When an attempt was made to isolate these organisms, they were found to be always accompanied by other bacteria, largely two spore-forming types. Keutner (28) concluded in 1905 that nitrogen-fixing bacteria are normal inhabitants of the sea, having been demonstrated in the North Sea, in the Indian Ocean, on the African coast, and in the Malay Archipelago. The amount of nitrogen fixed per culture containing 4 grams of glucose in 200 cc. of water varied from 5.7 to 16.0 milligrams, in the presence of calcium carbonate, and from 0.9 to 9.0 milligrams in its absence.

Keding (27) found *Azotobacter* to be present in the slimy substance formed on the surface of various marine algæ; he believed that the strains of this organism found in the sea are identical to those present on land. Nathanson (37), however, could not find *Azotobacter* in the Gulf of Naples; as a result of this, he was inclined to conclude that the occurrence of this organism in the sea is merely a matter of infection with land water. Benecke (6) repeated this work and had no difficulty in establishing the common occurrence of this organism in the Gulf of Naples; the negative results obtained by Nathanson were due entirely to improper methods of study. As is usually the case, negative results are frequently no proof at all that a specific organism is absent in the sea, but may be due to a lack of a proper technique or to unfavorable conditions of culture. Typical *Azotobacter* cultures were isolated by Benecke from mud taken at a depth of 20 to 100 meters.

The fact that *Azotobacter* occurs abundantly on marine algæ led Reinke (40, 41) to suggest that one deals here with a case of symbiosis, similar to that of leguminous plants, the algæ supplying *Azotobacter* with carbohydrates as sources of energy and using the nitrogen fixed by the latter. Issatchenko (24, 25) also demonstrated the presence of typical strains of *Azotobacter* and *Clostridium* in media inoculated with some fresh *Fucus*. When the medium was free from salt no development took place, tending to indicate that one deals here with organisms specifically adapted to sea water. The presence of both *Azotobacter* and *Clostridium* was established by Bavendamm (4) in the calcareous mud of various parts of the Bahama region, near Williams Island.

These results lead one to the conclusion that nitrogen-fixing bacteria are present in the sea, especially in the bottom material and in connection with the phytoplankton and marine algæ. Since these bacteria require a source of energy for their development and since the marine humus is not available for this purpose, one would naturally expect to find them in places where energy is made available either through the growth of the chlorophyl-bearing forms of life or after their death.

Experimental

The following experiments deal largely with the methods to be used for demonstrating the presence of nitrogen-fixing bacteria in the sea. In all cases the organisms must be allowed to develop on a favorable enrichment medium, containing an available source of energy and the necessary minerals which are either lacking in sea water or are present there in limited amounts. A presumptive test for the presence of non-symbiotic nitrogen-fixing bacteria in the enriched culture is the demonstration, by means of microscopic preparations, of the characteristic cells of the ærobic *Azotobacter* or the anærobic *Clostridium pastorianum.* Confirmative tests are made by determining the increase in total nitrogen content of the medium; for full confirmation, however, the organism must be isolated in pure culture and its ability to increase the total nitrogen content of an artificial medium determined.

The first experiment was largely of a preliminary nature, namely on the influence of the energy source upon the development of nitrogen-fixing bacteria in sea water media. One hundred-cc. portions of fresh sea water containing 0.05 per cent of K_2HPO_4 were placed in a series of flasks and 1 per cent quantities of various energy sources added. Some of the flasks received 40 grams of moist sand and others did not. When glucose and mannitol were used as sources of energy, abundant gas formation and butyric acid development took place, within 4–10 days in the flasks containing sand; this pointed to the development of anærobic butyric acid bacteria. The sand-free flasks gave some growth, which was not typical, however, of nitrogen-fixing bacteria. In view of the fact that no $CaCO_3$ was added to the medium and that the reaction was changed to pH 5.4–4.4, due to acid production, the lack of *Azotobacter* development could be readily understood. When calcium acetate was used as a source of energy, active development of the anærobic *Clostridium* was accompanied by the growth of *Azotobacter;* the final reaction of this medium was pH 7.4. Sterilization of the medium followed by inoculation with fresh sea water did not influence the development of the nitrogen-fixing organisms.

In view of the importance of iron in the process of fixation of nitrogen by *Azotobacter,* an iron-ligno protein compound (49) was also added. The final basic medium adopted for these investigations consisted of:

1000 cc. sea water
0.5 gram K_2HPO_4
0.5 gram iron-ligno-proteinate
20 grams glucose, mannitol, or Ca acetate.

The purpose of the next experiment was to establish the ability of the mixed bacterial population of the sea to fix nitrogen. One hundred cubic centimeter portions of the above medium, prepared from fresh sea water, were placed in a series of 250-cc. Erlenmeyer flasks. Some of the flasks received 40-gram portions of sand, 2–3 grams of $CaCO_3$ and 2 grams of the energy source; some flasks received only $CaCO_3$ and the energy source, while some did not receive any $CaCO_3$. The cultures were incubated for 32 days and the nitrogen determinations made.

The results presented in Table VII prove that sea water contains active nitrogen-fixing organisms. The presence of sand is not necessary to bring about their optimum activities. Glucose and mannitol were found to be usually better sources of energy than Ca acetate. The addition of $CaCO_3$ is necessary, when sugars are used, to neutralize the acidity produced by the anærobic organisms; the presence and activities of these are indicated by the abundant gas evolution. The crude cultures fixed appreciable quantities of nitrogen. The fixation of nitrogen was greatest in the presence of $CaCO_3$, except in the case of those cul-

TABLE VII

Nitrogen fixation in sea water with and without sand, with different carbon sources. Two grams of energy source used per culture. Cultures were incubated 32 days.

Sand present	Energy source	$CaCO_3$ added	Gas formation	Nitrogen fixed per culture
				mg.
–	Glucose		0	11.2
–	Glucose	+	0	12.5
–	Mannitol		+	0.2
–	Mannitol	+	+	13.8
–	Ca acetate		+	0.9
–	Ca acetate	+	Trace	2.0
+	Glucose		+	3.7
+	Glucose	+	0	8.2
+	Mannitol		+	4.8
+	Mannitol	+	0	8.6
+	Ca acetate		0	3.1
+	Ca acetate	+	0	4.6

tures where lack of gas evolution pointed to the unfavorable conditions for the development of the anærobic bacteria; a good pellicle development of *Azotobacter* and accompanying forms always indicated abundant fixation of nitrogen.

In order to test the presence and distribution of nitrogen-fixing bacteria in the sea, two types of media were used, one favorable for the development of anærobic bacteria and one for the growth of ærobic organisms: 1. The medium previously described was prepared, and 35-cc. portions, together with 10 grams of dry washed sand, were placed in large test tubes and sterilized for 15 minutes at 15 lbs. pressure; this medium was particularly favorable for demonstrating the presence of the anærobic *Clostridium* in sea water and in sea bottom; the relative abundance of this organism was determined by the dilution procedure. 2. The silica-gel plate was utilized for the purpose of demonstrating the presence of ærobic nitrogen-fixing bacteria in the sea.

The plates containing the gel (47) are placed in flowing tap water for 12–16 hours, to remove excess of chloride; they are then removed from the tap water and immersed in freshly boiled and cooled sea water, for 5–10 minutes. The excess of water is drained off and carefully dried off and the plates covered with sterile tops. A nutrient solution[4] is prepared so that when 2 cc. of this solution is added to a 100-cc. portion of silica gel, using a plate 15 cm. in diameter, the final medium will contain 2 per cent of the carbon source. Distilled water is used for the preparation of the nutrient solution. The plates are then exposed in a drying oven kept at 60° C. for a few minutes, until the excess of water has been removed. A thin layer of sterile $CaCO_3$ is powdered on the surface of the medium, the plates are covered with sterile tops, and are ready for inoculation. Special care must be taken in the preparation of this medium, since an excess of salt or too thin a layer of medium will result in the rapid drying out of the gel and will prevent bacterial development.

The cruise of the " Atlantis " in August, 1932, to the Gulf of Maine and George's bank (50) presented a good opportunity for determining the presence of ærobic and anærobic nitrogen-fixing bacteria on the high seas, both in the sea water and in the mud. A series of tubes with sterile liquid medium and a series of plates with the silica-gel medium were taken aboard ship and inoculated with water and mud from the various stations, immediately after the samples were taken. It was demonstrated in a previous contribution (50) that there is a definite parallelism between the total numbers of bacteria in the water, as determined by the plate method, and the number of plankton organisms;

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[4] This solution also contained 0.05 per cent K_2HPO_4 and 0.05 per cent iron lignoprotein complex.

this was especially true of the phytoplankton. Whether the occurrence of *Azotobacter* in the sea has also a certain relation to the phytoplankton still remains to be determined. At the time of the cruise, there was comparatively little phytoplankton in the water. This may offer the explanation for the fact that not all the stations, especially the free water, gave evidence of *Azotobacter* growth. It may also be due to the fact that the silica gel medium used in this experiment was not quite favorable for the growth of the organism, as found later when the details of the method were better understood.

In the case of one station (1329), the surface layer of the marine mud gave an excellent growth of the typical *Azotobacter chroöcoccum,* using the silica gel plate. The liquid cultures showed marked differences, when water and mud were used as inocula. When 1 cc. of surface water was added to the medium, good pellicle development took place; the pellicle contained typical *Azotobacter* cells, which were also accompanied by various other bacteria. In the case of one station (1331), samples of water were taken, by means of sterile glass tubes, at the surface of the water and at depths of 30, 50, 100, and 215 meters. Only the surface and the 50 meter samples gave pellicle formation on the liquid medium; the other samples gave no signs of growth. Very few of the samples of free sea water, obtained from the different stations, brought about any gas formation or butyric acid production in the liquid medium, thus proving the absence of the anærobic *Clostridium* in the free water.

TABLE VIII

Fixation of nitrogen by crude culture of Azotobacter chroöcoccum. Two-gram energy source.

Energy source	Organism	Medium	Incubation	Nitrogen fixed per culture
			days	*mg.*
Glucose	Control	Silica plate	21	0.1
Glucose	Azotobacter	Silica plate	21	5.4
Mannitol	Azotobacter	Liquid medium	15	6.4
Glucose	Azotobacter	Liquid medium	17	4.8

The liquid media inoculated with the mud samples gave abundant gas formation, the medium itself becoming decidedly turbid. A microscopic examination of the cultures showed the presence of *Clostridium* cells, especially when glucose was used as a source of energy. This organism was always accompanied by other bacteria, usually small and large rods, some of which were spore-forming organisms.

These results suggest that anærobic nitrogen-fixing bacteria of the

Clostridium pastorianum type are universally distributed in the sea bottom, but are present only to a very limited extent in the sea water The ærobic *Azotobacter* is also found in the sea, but much less frequently than the anærobic *Clostridium.* Whether *Azotobacter* is less abundant in the sea or whether the media used for demonstrating its presence were not always sufficiently favorable for its development remains to be determined. Although there is no doubt that *Clostridium* finds its natural medium in the sea bottom, it still remains to be established whether *Azotobacter* is normally present in the sea water, in the plankton, on the surface of algal growth, or in the sea bottom. Benecke and Keutner, Issatchenko, and others found this organism on the surface of marine algæ and suggested, therefore, its symbiotic existence with the latter; however, its presence on the bottom of the sea suggests also the possibility of its free existence, whereby the remains of algal and plankton growth are used as sources of energy.

Various other bacteria were encountered in the nitrogen-free media, either accompanying *Clostridium* and *Azotobacter,* or even without these organisms; they consisted of different cocci and small rods, occasionally thread-like forms. Whether these bacteria are able to fix nitrogen or whether they play any part in this process at all still remains to be determined.

In order to demonstrate the ability of the culture of *Az. chroöcoccum* obtained from the bottom mud of Station 1329, by the use of the glucose-silica-gel plate, to fix atmospheric nitrogen, the total nitrogen content of the gel in which this organism was growing was compared with that of a control plate. The crude culture of this organism was also inoculated into a 100-cc. portion of liquid medium containing 2 per cent of glucose or mannitol, incubated for 15–17 days and the amount of nitrogen fixed determined (Table VIII). The results obtained prove beyond any doubt the ability of the *Azotobacter* found in the sea bottom to fix considerable quantities of nitrogen in sea water media, in the presence of available sources of energy.

The *Azotobacter* was later isolated free from the accompanying bacteria, as shown in Fig. 3. However, in view of the fact that the nitrogen-fixing organisms do not live in the sea in the form of pure cultures, but are present there as mixed populations, a knowledge of their ability to fix nitrogen in such a mixture is more important than that of a pure culture.

Figures 1 to 8 show a group of preparations of bacteria isolated from the nitrogen-fixing cultures in which sea water and bottom material of the Gulf of Maine were used. The *Azotobacter* produced on the silica gel plate a typical growth of *Az. chroöcoccum,* forming at first a yellow pigment which later turned deep brown to black. The *Clostridium* produced abundant gas from sugars in liquid media. The ability of the three small bacterial forms shown in Figs. 6, 7, and 8 to fix nitrogen has not been determined. They are common types which develop on nitrogen-free media inoculated with sea water or with sea bottom material.

Although the results presented here are to be considered as preliminary in nature, they are quite sufficient to bring out the fact that nitrogen-fixing bacteria, both of the ærobic and anærobic types, are commonly found in the sea. There is no doubt that in the presence of an available source of energy and other nutrients, such as available iron and phosphate, they become active in the sea and are able to fix atmospheric nitrogen.

A study of the relation of these bacteria to the phytoplankton and plankton residues on the sea bottom, the natural sources from which they derive their energy, would necessitate a study of the occurrence of *Azotobacter* at certain stations throughout the year. This, as well as further study of the relation of the nitrogen-fixing bacteria to the available nutrients in the sea, changing conditions, season of year, depth, distance from shore, etc., are still problems awaiting further investigation. For this purpose, the silica gel plate method seems to be ideal for the determination of the occurrence of *Azotobacter,* while the liquid-sand culture in the tube is very convenient for studying the abundance of *Clostridium.*

SUMMARY

1. A preliminary study has been made of the occurrence and activities in the sea of bacteria concerned in the cycle of nitrogen in marine life.

EXPLANATION OF PLATE I

FIG. 1. Mixed culture of *Azotobacter* and accompanying bacteria. Mud of Station 1329 inoculated on silica gel plate. × 1031.

FIG. 2. Culture of *Azotobacter* on liquid media, isolated from sea water, accompanied by short rod-shaped bacteria. × 1031.

FIG. 3. Pure culture of *Azotobacter* isolated from sea water. × 1031.

FIG. 4. Culture of *Azotobacter* isolated from mud of Station 1329 on silica-gel media and cultivated on nitrogen-free glucose media, showing granulation. × 1031.

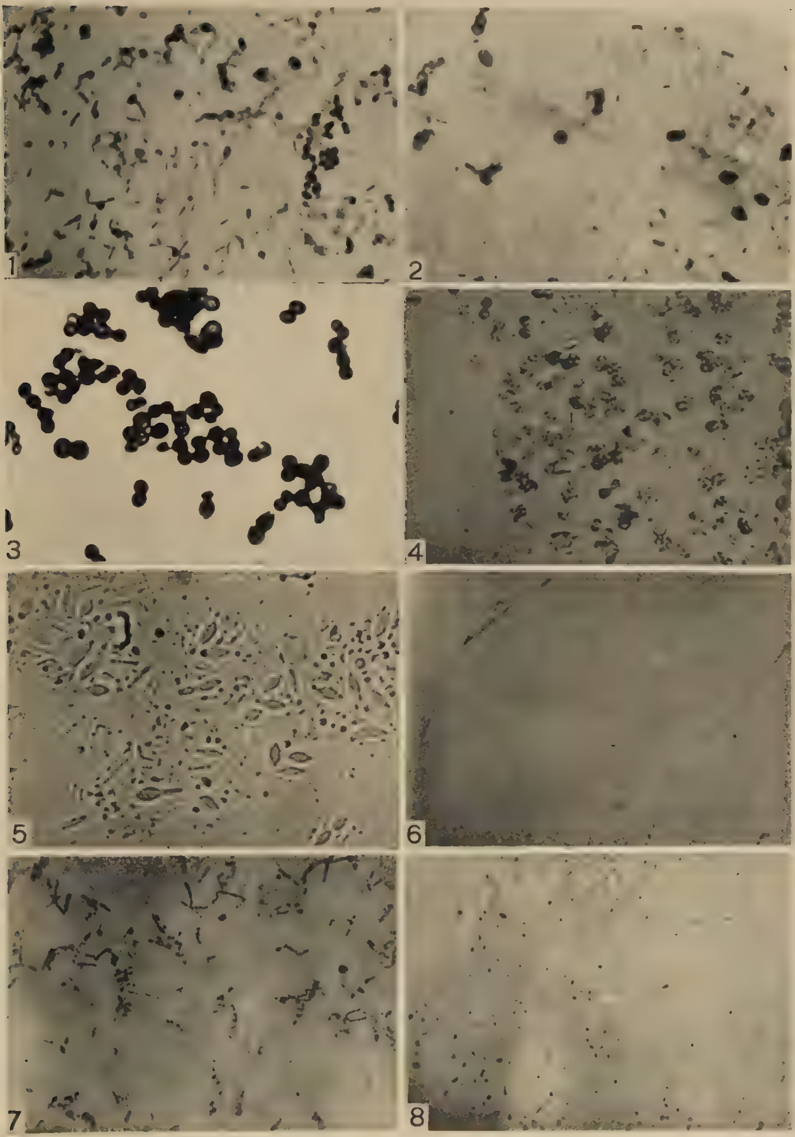
FIG. 5. Culture of *Clostridium* isolated from marine mud. Culture impure, unstained preparation. × 1031.

FIG. 6. Thread-forming bacteria growing in nitrogen-free glucose media inoculated with marine mud. × 1031.

FIG. 7. Spore-forming rod-shaped bacteria growing on nitrogen-free media. × 1031.

FIG. 8. Spore-forming rod-shaped bacteria isolated from surface water in the Gulf of Maine (Station 1331) and grown on nitrogen-free mannitol media. × 1031.

Plate I



2. Sea water, especially in the upper layers, is practically free from nitrifying bacteria or contains only very few cells. The activities of these organisms are limited almost entirely to the sea bottom.

3. In order to demonstrate the presence of these organisms even in the bottom material, it is essential to use a medium containing a layer of sand or mud, so as to make conditions favorable for their development.

4. It is comparatively easy to demonstrate the presence in the sea bottom of bacteria oxidizing ammonium salts to nitrite. It is somewhat more difficult to establish the presence of nitrate-forming bacteria; this can be done, however, if the sensitiveness of these organisms to the laboratory conditions is taken into consideration, especially nutrient concentration and period of incubation.

5. Sea water and sea bottom contain an abundance of nitrate-reducing bacteria. Most of these organisms, especially those present in the water, are able, however, to reduce the nitrate only to nitrite, but not to atmospheric nitrogen.

6. Bacteria that bring about complete reduction of the nitrate are present largely in the sea bottom. In order to reduce the nitrate, an available source of energy must be present. The marine humus cannot be used to any great extent as such a source of energy. The plankton residues are used largely by those bacteria which consume the nitrate and transform it into organic nitrogenous compounds.

7. The conclusion is reached that, even if denitrifying bacteria are present in the sea, their activities are so limited under marine conditions as to render them in most instances of little importance in limiting the nitrate supply of the sea.

8. The sea contains an abundant population of nitrogen-fixing bacteria. The ærobic *Azotobacter* is largely found in the water and in the surface layer of the marine bottom, while the anærobic *Clostridium* is present in the sea bottom.

9. In the presence of a favorable source of energy, the marine nitrogen-fixing bacteria are capable of fixing appreciable quantities of nitrogen. It still remains to be determined, however, to what extent this process actually takes place in the sea.

10. The bacteria concerned in the transformation of nitrogen can be tentatively distributed among the plankton and the sea bottom, the two major seats of bacterial activities in the sea, as follows: the nitrifying bacteria live largely on the sea bottom; of the nitrate-reducing bacteria, those forms which reduce nitrate to nitrite live both in the water and in the bottom, while the true denitrifying bacteria live largely on the bottom; of the nitrogen-fixing bacteria, the ærobic forms live both in the

water and on the bottom, while the anærobic forms live largely in the bottom material.

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MARINE BACTERIA AND THEIR RÔLE IN THE CYCLE OF LIFE IN THE SEA

MARINE BACTERIA AND THEIR RÔLE IN THE CYCLE OF LIFE IN THE SEA

III. The Distribution of Bacteria in the Ocean Waters and Muds About Cape Cod[1]

HERBERT W. REUSZER[2]

Introduction

A considerable number of investigations concerning the number of bacteria and their distribution in sea water have been made. Among the earliest of these was that of Russell (1892, *a* and *b*, 1893), who conducted systematic studies of the distribution of bacteria in the Gulf of Naples. He found bacteria in small numbers to be uniformly distributed in the water at distances of 3 to 15 kilometers from shore and at depths ranging from 50 to 1,500 meters. It was found by others (Cassedebat, 1894; de Giaxa, 1899) that while the water of harbors and bays might contain large numbers of bacteria, this number, as determined on the ordinary solid bacteriological media, became very small at comparatively short distances from shore. Bassenge and Fischer (1894, *a* and *b*), who investigated the water over a wide area of the Atlantic Ocean, found bacteria in larger numbers than have any other investigators. The largest numbers, however, were found near land or in the presence of large quantities of floating sea weed. Lloyd (1930), in her investigations in the Clyde Sea area, followed the distribution of bacteria at five stations during the course of a year. In the rather shallow waters of this region, the surface water was found to be richest in bacteria, the number decreasing with depth but usually increasing slightly again at the bottom. The numbers in the surface water fluctuated considerably but no definite seasonal trend could be detected; below the surface the numbers were remarkably constant throughout the year. Numerous, usually somewhat fragmentary investigations, ranging from polar to tropical waters, have almost invariably shown small numbers of bacteria to be present in the sea water. Among these may be cited those of Frankland and Burgess (1897), Levin (1899), Otto and Neumann (1904), Gazert (1912), Berkeley (1919) and Lipman (1929). The results of recent investigations such as those of Cholodny (1929) and Rasumow (1932), in the direct microscopic examination of sea water, indicate that bacteria are much more abundant than the cultural methods used by previous investigators have shown.

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[2] The author wishes to acknowledge the assistance of Mr. Charles E. Renn in certain of these experiments.

Studies concerning the bacteria of marine bottom deposits have been few. Russell (1892 *a*) studied the distribution of bacteria in the mud of the Bay of Naples simultaneously with his studies of bacteria in the water. This included mud at depths ranging from 50 to 1,100 meters below the surface of the water and extending to 15 kilometers from land. He found that down to depths of 200 meters, the number of bacteria decreased with distance from land, the range being from 70,000 to 285,000 bacteria per cubic centimeter of mud. Below 200 meters the number remained more constant. He had no satisfactory explanation to offer for this distribution. In work of the following year (1892 *b*) on the mud of Buzzards Bay on the Massachusetts coast, he found smaller numbers of bacteria than at Naples, a fact which he correlated with the lower water temperature in Buzzards Bay. The number of bacteria in sand bottom was much lower than in mud. Russell considered the mud to have a bacterial flora indigenous to itself, since two or three species of bacteria normally found only in the mud made up 30 to 50 per cent of all the bacteria present. Fischer (1894 *a*) and Gazert (1912) appear to be the only investigators who have studied the bacteria in deep sea deposits. Neither found bacteria to be very abundant in deposits of this nature. From many of their samples no bacteria developed. Their results are in direct contrast with those obtained from marine mud near shore. In the chalky mud of the flats about Andros Island in the Bahamas, Drew (1913) found bacteria to be present up to the number of 160,000,000 per cubic centimeter. Lipman (1929) and Bavendamm (1932) likewise found very large numbers of bacteria in these deposits. Lloyd (1931), in her systematic investigations in the Clyde Sea area, found bacteria up to 300,000 per gram of dry mud in the surface layer of mud. The number of bacteria decreased with depth and in the lower depths of the mud remained fairly constant. In the upper layers of mud there was in certain cases a bacterial zonation which was probably correlated with the stratification of the mud into organic and inorganic layers as noted by Schokalsky (1928) and Moore (1931).

The literature of marine bacteriology has recently been reviewed extensively by Benecke (1933), but the brief review above is sufficient to indicate that the work in general has been fragmentary in nature. There have been few studies of the distribution of bacteria in the sea in which an attempt was made to establish definitely the significant factors with which the observed changes in bacterial numbers might be associated. The supplementary data obtained by various investigators is in most cases so scanty as to make it impossible to correlate the results of one investigator with those of another.

The studies reported below represent preliminary results in a more extensive study of the occurrence of bacteria in the sea and the factors influencing their distribution. The areas investigated were the continental slope south and east of Cape Cod, the Gulf of Maine, and certain of the bays adjacent to Cape Cod. The station locations, with the exception of those in Buzzards Bay and the Gulf of Maine, are given in Fig. 1. The location of the Gulf of Maine stations has been previously given (Rakestraw, 1933).

EXPERIMENTAL

Methods

The water-sampling bottle used in this work was similar to the evacuated glass tube used by Russell (1892 *a*), the original idea of which is ascribed by him to Massea. For breaking the tip of the inlet tube at the proper depth, a modified form of the sampler described by Wilson (1920) was used. Mud samples were obtained by means of a modified form of the sampler described by Moore and Neill (1930). The glass tube in which the sample was secured was sterilized before use and the sample for analysis taken from the center of the core. The tube was closed at both ends with a sterilized rubber stopper immediately upon reaching the surface. No sampler satisfactory from the bacteriological standpoint is available for sampling sandy deposits. The few analyses of the bacterial content of such deposits reported in this paper were obtained from samples taken from the center of the material brought up in a sampler of the clam shell or snapper type.

In the present investigation, as in most previous ones, the plate method of determining the number of bacteria was used. It should be pointed out that since not all bacteria are capable of developing on the agar plate, the results so secured must be interpreted with considerable caution, particularly in regard to the total number of bacteria found. The observations of Pütter (1924), based on the oxygen consumption of the bacteria in sea water, and the work of Cholodny (1929) and Rasumow (1932) with the direct microscopic count, all indicate that the number of bacteria actually present in sea water is much greater than the cultural methods of determining their abundance have indicated. In preliminary experiments with various media, one having the following composition gave the highest counts of bacteria and was used throughout the work:

Peptone	1 gram
Glucose	1 gram
K_2HPO_4	0.05 gram
Agar	15 grams
Sea water	1000 cc.

It is interesting to note that with the exception of the addition of glucose, this medium is similar to the one employed by Drew (1913) and which Lipman (1929) found to give higher counts than any of the other media with which it was compared. Sea water was plated both undiluted and diluted ten times, three plates of each dilution being used. The mud was plated in a similar manner, except that the moist mud was diluted 10, 100, and 1000 times. Moisture determinations were made on the mud samples and the results are reported as number of bacteria per gram of dry mud. All samples, immediately upon being taken, were placed in a refrigerator at 4–5° C. and kept at this temperature until plated. In most cases the plating took place in less than twenty-four hours after taking the sample. The plates were incubated at room temperature and counted at the end of 7 and 10 days.

Abundance of Bacteria in Sea Water

In order to determine the variability which might normally be expected in the number of bacteria in sea water, 10 samples were taken at a single station, on the 20-fathom line south of Martha's Vineyard. All the samples were taken at a depth of 10 meters, and were plated out immediately when brought to the laboratory. The number of bacteria found in each of the 10 samples was as follows: 6, 2, 3, 4, 8, 83, 10, 24, 11, 27, 18. The average number of bacteria for all the 10 samples was 19 per cubic centimeter, with a variation between the individual samples of 2 to 83 cells per cubic centimeter. It would seem, therefore, that considerable variation may be expected in the number of bacteria in samples of sea water taken from the same spot. Russell (1893) observed that an occasional sample of sea water contained much larger numbers of bacteria than was to be expected from the bacterial content of the particular locality. A single species of bacteria always predominated in such samples. While the number of colonies appearing on plates of the same series was in general quite uniform, a similar observation was made during the course of the present work. The increased number of organisms sometimes was very large and occasionally appeared on only one plate of a series. Russell explained this phenomenon by assuming that some of the bacteria in sea water exist in the form of zoögleal masses. It suggests also, however, that part of the bacteria may be associated with particulate matter in the sea and that the large numbers of bacteria in certain samples may be due to the presence of the decomposing remains of some small marine organism such as a copepod or a chain of diatoms.

The data in Table I give an indication of the number of bacteria occurring in water receiving land and sewage drainage and illustrate the effect of local currents. Samples 1 and 2 were taken in the outer harbor of New Bedford and were very much higher in bacteria than were the three samples taken in Buzzards Bay outside the harbor. The location of these stations is not indicated on Fig. 1, but none of them were more than one or two miles from land. At the time the samples were taken the tide was rising and the direction of water movement was from Station 5 into the harbor towards Stations 1 and 2 and up the bay from Station 5 towards Station 3. The fact that the water, taken so near a source of pollution as was Sample 1, did not contain more bacteria is probably due to the fact that with an incoming tide at this time the tidal currents tended to carry the polluted water up into the harbor and left in its place the Buzzards Bay water from Station 5 with relatively low bacterial content. This is indicated by the fact that the water at Station 1, 100 yards on the bay side of the sewer outlet, contained 3,200 bacteria per cubic centimeter, while that at Station 2, approximately one mile away but in the direction of the water movement, contained 4,200 per cubic centimeter. The water in the bay itself contained much smaller numbers of bacteria. At Stations 4 and 5 there were about 100 bacteria per cubic centimeter, while

TABLE I

Abundance of bacteria in water and mud of New Bedford Harbor and Buzzards Bay, July 1, 1932.

Station	Location	Water depth	Number of bacteria per cc. of water or per gram of dry mud				Carbon content of surface mud	Carbon content of mud at 10 cm. depth
			Surface water	Bottom water	Top 2.5 cm. of mud	Mud at 10 cm. depth		
		meters					*per cent dry mud*	*per cent dry mud*
1	100 yds. from sewer outlet	9	1790	3230	158,500	20,100	3.37	1.21
2	Across channel from sewer outlet	8	—	4250	41,200	—	0.58	
3	Above harbor entrance	15	—	197	79,900	—	2.30	
4	Directly opposite harbor entrance	10	—	94	48,000	44,400	1.96	
5	Below harbor entrance	18	22	111	34,000	—	1.76	

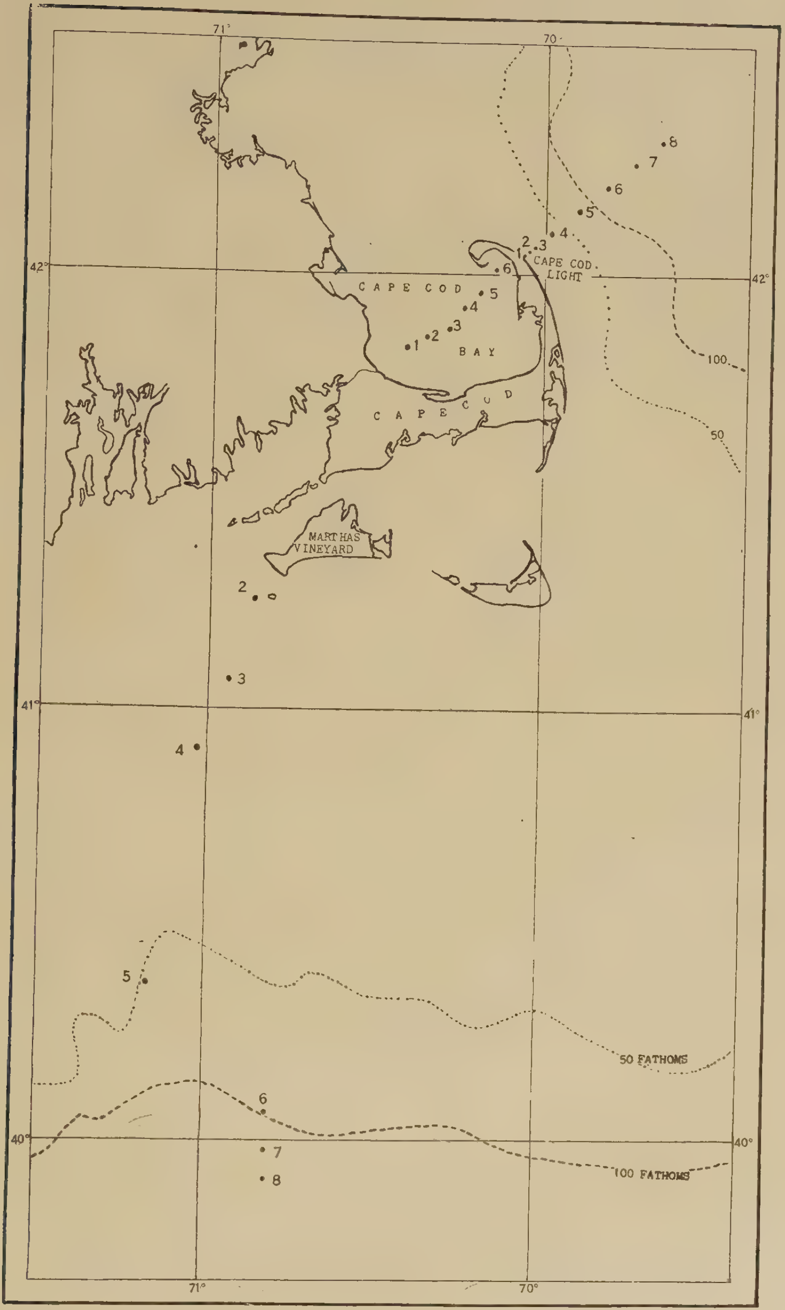
71°
70°
42°
41°
40°
CAPE COD BAY
CAPE COD
CAPE COD LIGHT
MARTHAS VINEYARD
100
50
50 FATHOMS
100 FATHOMS
1
2
3
4
5
6
7
8

FIG. 1. Chart showing location of the stations sampled for determination of the number of bacteria in sea water and bottom deposits.

at Station 3 farther up the bay the number reached 200 per cubic centimeter. The surface water evidently contained fewer bacteria than did the bottom water. An incomplete series of surface samples was secured because of loss of vacuum by the bottles prepared for taking them.

To secure information as to the number of bacteria in water and bottom deposits of the open ocean away from the influence of land a line of stations was sampled from the southern shore of Martha's Vineyard Island to the continental slope about 80 miles to the south. The results of these determinations are given in Table II. The number of bacteria in the surface water was found to vary irregularly and did not seem to be correlated with distance from land or depth of water. While the sample taken at 8:30 in the morning contained the highest number of bacteria, the sample with the second highest number of bacteria was taken at 6:00 P.M. There appeared to be no definite correlation between bacterial numbers and the length of time during which the surface water had been exposed to sunshine, as was suggested by Fischer (1894 *a*). The bottom water at the two stations at which samples were obtained was much higher in bacteria than the corresponding surface water. These samples were taken very near the bottom in water of no great depth, where agitation of the surface of the bottom by water movements may have carried bacteria into the lower layers of water.

TABLE II

Abundance of bacteria in sea water and in sea bottom deposits south of Martha's Vineyard, July 19–20, 1931.

Station No.	Depth of water	Distance from land	Hour of day when sample was taken	Number of bacteria per cc. of water or per gram of dry mud				Loss on ignition
				Surface water	Bottom water	Top 2.5 cm. of mud	Mud at 10 cm.	
	meters	*miles*						*per cent dry mud*
2	18	1.5	7:00 P.M.	65	159	—	—	—
3	38	12	5:00 P.M.	183	1900	238 (sand)	—	0.69
4	55	23	3:30 P.M.	185	—	1050 (sand)	—	0.94
5	94	55	8:30 A.M.	735	—	7700	2700	7.93
6	163	72	7:00 P.M.	27	—	1000 (sandy)	—	3.17
7	345	77	6:00 P.M.	480	—	1400	460	6.14
8	570	81	3:00 P.M.	190	—	1280	900	7.24

A series of observations of the bacterial content of the water and mud off the eastern coast of Cape Cod north of the Nantucket Shoals and Georges Bank was made during the summer of 1932. This line of stations extends NE by E$\frac{1}{2}$E from Cape Cod Light, the sampling stations being 100 yards and 1, 2, 5, 10, 15, 20, and 25 miles from shore. As will be seen from the water depths, the ocean bottom along this line slopes gently downward to about 15 miles off shore, where it levels off at a fairly constant depth of about 245 meters. The number of bacteria found in the water of these stations at three different periods during the summer is recorded in Table III. With the exception of the samples taken near the beach, extremely few bacteria were present in the water at any time. A considerable number of the samples did not contain any bacteria capable of developing on the agar plate. The highest number found was 78 per cc. at Station 4 on August 24. The number of bacteria tended to be slightly higher at all stations at this date. The number of bacteria in water one mile from shore was no greater than in water taken farther from shore. On an exposed shore such as this with the sea receiving almost no land drainage, it is doubtful if the number of bacteria in the water is directly affected by the presence of land. The larger number of bacteria in water from the beach is no doubt due to bacteria carried up from the bottom by wave action.

TABLE III

Abundance of bacteria in sea water off Cape Cod Light, July 13–September 20, 1932.

Station No.	Distance from shore	Depth of water	Number of bacteria per cc. water					
			July 13		August 24		September 20	
			Surface water	Bottom water	Surface water	Bottom water	Surface water	Bottom water
	miles	*meters*						
1	100 yds.	5	38	2	394	793	—	—
2	1	25	0	1	—	—	—	—
3	2	36	0	0	—	—	—	—
4	5	83	1	0	78	22	—	—
5	10	159	3	0	1	1	—	—
6	15	243	0	1	12	3	0	—
7	20	246	4	0	5	—	0	1
8	25	245	—	—	5	2	0	0

The bacteria in the water off Cape Cod Light may be compared with the number found in Cape Cod Bay (Table IV) and also with those for the Gulf of Maine and Georges Bank. The latter results have been discussed elsewhere (Waksman et al, 1933) in connection with other biological data secured at the same time. The water taken from the

Gulf of Maine proper, Stations 1329–1333, was, with a single exception, characterized by low numbers of bacteria similar to those found in the water off Cape Cod Light. The surface water at Station 1333 contained the exceptionally high number of 380 bacteria per cubic centimeter. No explanation for this is known except the possibility of the presence in the sample of the decaying remains of some small marine organism. The number of bacteria in the relatively shallow water over Georges Bank, Stations 1334–1336, was uniformly higher than in the Gulf of Maine. This paralleled a higher phytoplankton content in the Georges Bank water (Gran, 1933). The finding (Waksman et al, 1933) that large numbers of bacteria occur with the plankton, particularly the phytoplankton, suggests that the higher bacterial content of this water is due to increased available organic matter resulting from the high plankton content. Bacterial numbers did not vary significantly with depth at any of these stations. If the above explanation relating an increased bacterial content with an increased phytoplankton content is correct, then it would seem that the numbers of the two types of organisms should have a parallel variation with depth. The studies reported above were not detailed enough to determine whether or not this was the case. It is possible that with the low quantities of plankton ordinarily found in the sea during the summer months, it would be very difficult to detect variations of bacteria with depth during this time. A study of the numbers of bacteria associated with the annual spring increase of the plankton should afford results extremely interesting from the standpoint of the decomposition and transformation of the organic materials produced in lreatively large amounts at that time.

TABLE IV

Abundance of bacteria in sea water of Cape Cod Bay, August 24–September 21, 1932.

Station	Depth of water	Number of bacteria per cc. water			
		August 24		September 21	
		Surface water	Bottom water	Surface water	Bottom water
	meters				
1	26	8	23	—	—
2	28	19	15	—	—
3	35	17	26	—	—
4	38	9	36	2690	850
5	37	8	21	4670	720
6	26	26	23	2620	1020

Returning to the water of Cape Cod Bay, it will be seen that the number of bacteria found there on August 24 is significantly higher than that found on the same date off Cape Cod Light but approximately equal to the number occurring over Georges Bank on August 5. At three of these stations sampled on September 21 there was an increase in the number of bacteria over that found on August 24 amounting to practically a hundred-fold in both the surface and bottom water. This increase may or may not be due to the fact that the samples of September 21 were taken between 4:00 and 6:00 A.M., while those of August 24 were taken from 7:40 to 11:25 P.M. On August 24 the bottom water at four of the six stations contained more bacteria than did the surface water. Of the three stations sampled September 21, all contained from two to six times as many bacteria at the surface as at the bottom. The only factor under observation which varied at the two sampling periods was, as noted above, the time of day at which the samples were taken. This does not seem to offer an adequate explanation for the wide variation found in the distribution of the bacteria.

Abundance of Bacteria in Sea Bottom Deposits

The mud samples of which the bacteriological analysis is reported here were obtained at the same stations as those described above for the water samples. In general no attempt was made to sample the bottom deposit at those stations where it consisted only of sand. At a few stations, however, sand samples were obtained and these are designated as such wherever they occur in the tables. The deposits examined were entirely of terrigenous nature. The material from which the bacteria living in the bottom deposits of this type secure their food comes then from two sources: (1) The organisms living in or on the sea bottom and those sinking from the water above, (2) the organic matter present in a partially decomposed state in the sediments brought in from land. The relative amount of these two sources of organic matter will vary widely depending upon local conditions. The fresher organic matter tends to accumulate at the surface of the bottom and to become incorporated into the lower layers of mud only very slowly through the action of burrowing organisms living in the marine bottom. Sand bottoms contain relatively little organic matter, but the findings of Raymond and Stetson (1931) show that over them also there occurs a small amount of organic material.

The number of bacteria found in the ocean bottom at the stations south of Martha's Vineyard in July, 1931, is given in Table II. The figures for loss on ignition of the dry mud are also given as a rough indication of the amount of organic matter in the samples. The sand samples contained fewer bacteria than did the mud samples. This is especially true of the sand taken nearest shore where there is practically no organic matter present. In the mud samples there is a progressive diminution in the number of bacteria present with distance from shore. The number of bacteria in these samples cannot be definitely correlated with the amount of organic matter present, probably because in these very finely divided sediments a considerable part of the loss on ignition is due to the loss of physically or chemically bound water. The number of bacteria diminished rapidly with depth into the mud. Later determinations on mud samples from the Gulf of Maine indicated

TABLE V

Abundance of bacteria in sea water and in bottom deposits of the Gulf of Maine and Georges Bank, August 2–5, 1932.

	Number of bacteria per cc. water							
Station No.	1329	1330	1331	1332	1333	1334	1335	1336
Depth of water in meters	256	207	230	346	230	71	74	64
Depth of sample								
Surface	10	3	4	2	380	16	92	6
50 meters	1	1	2	1	2	—	—	—
Bottom	6	0	1	1	3	25	41	27
Depth in mud	Number of bacteria per gram of dry mud or sand							
Surface 2.5 cm	5400	4700	830	3000	—	—	1280 (sand)	670 (sand)
10 cm	1720	1700	140	290	—	—	—	—

(Table V) the fact that they had a bacterial content of the same order of magnitude as the muds south of Martha's Vineyard. The figures for the number of bacteria in mud samples from Buzzards Bay and New Bedford Harbor given in Table I indicate the much higher bacterial content of sediments near land. The carbon content of these muds as determined by a modification (Tiurin, 1931) of the method of Schollenberger (1931) is given also. The determinations indicate a very close direct correlation between the number of bacteria in these samples and the content of organic matter as indicated by the carbon determination.

In the sampling method for mud, described above, the mud was obtained as a core in a glass tube. The core was removed from the tube by pouring off the water above the mud, inserting a small piece of sterile cotton, and then pushing the mud from the tube with the aid of a wooden rod slightly smaller in diameter than the inside diameter of the glass tube. By this procedure the surface layer of mud became somewhat compressed and mixed and the extreme surface was absorbed to some extent by the cotton. The surface sample of mud represented, therefore, a composite of approximately the top inch of mud. The organic matter added to the mud from the water above tends to accumulate at the very surface of the mud in a zone probably so thin as to be measured in millimeters rather than centimeters. A change in the procedure for removing the mud from the tube was then adopted in order to study the number of bacteria in the extreme surface layer of mud. In pouring off the water above the tube about 4 cc. were allowed to remain behind. Previous platings of this water had shown that unless mixed with mud very few bacteria were present. The tube was then shaken until the surface layers of mud were in suspension. One cubic centimeter portion of the suspension was then pipetted off for bacteriological analysis, another for moisture and total carbon determination, and another for a sea salt determination. The amount of dry material in one cubic centimeter of such a suspension was usually about 60 mgm., although it varied from 35 to 90 mgm. with different samples. Of the dry material, usually one-half or more, depending upon the concentration of mud in the suspension, was sea salt. The carbon and bacteriological determinations were corrected for the presence of this salt.

Table VI

Distribution of bacteria and organic carbon in the mud of the Atlantic Ocean off Cape Cod Light, August–September, 1932.

Station No.	Depth of mud	Bacteria per gram of dry mud							Per cent organic carbon in dry mud		
		August 24				September 20			September 20		
		Surface	2.5 cm.	10 cm.	20 cm.	Surface	2.5 cm.	10 cm.	Surface	2.5 cm.	10 cm.
6		5,000,000	630	340	—	125,500	4,300	270	5.20	1.30	1.37
7		919,000	1695	176	—	48,800	4,900	2,390	16.66	1.50	1.77
8		23,100	1490	220	0	25,300	4,700	2,290	5.77	1.72	1.77

The number of bacteria found in mud samples from Cape Cod Bay and the Atlantic Ocean off Cape Cod Light by the above method are given in Tables VI and VII. The carbon content of the mud is included in these tables. The carbon content of the surface layer was considerably higher than that of any of the lower layers. In two cases values as high as 16.66 and 20.00 per cent carbon in the surface mud were found, but these values may possibly be excessive. An inspection of this data shows at once the importance of the extreme surface layer of the mud from the standpoint of bacteriological activity. The number of bacteria in the surface exceeded by far the number present at a depth of only 2.5 cm. at all stations. Not only was the surface layer rich in total number of bacteria but it also contained a much more varied bacterial flora than did the lower layers. Agar plates of samples from the 2.5 cm. depth revealed only small, grayish white, either round or lens-shaped colonies. Plates from the surface layer, on the other hand, contained many types of colonies, including occasionally agar-decomposing forms. This surface layer of mud, having a high concentration of available organic matter and a source of available oxygen in the water above, appears to be the seat of the greatest activity of the marine bacteria. The number of bacteria in the surface mud varied markedly at the two sampling periods. However, the number of bacteria decreased on the ocean side of Cape Cod and increased correspondingly in the bay. It should be noted that, as shown in Table IV, the number of bacteria in the water of Cape Cod Bay also was markedly greater at this time than at the previous sampling period. It is generally assumed that there is an accumulation of sediment going on in Cape Cod Bay, part of this coming from the outside of Cape Cod. Whether there is any relation between this deposition of fresh sediment and the observed change in the bacterial content of the mud is uncertain. The fluctuation of the bacterial

Table VII

Distribution of bacteria and organic carbon in the mud of Cape Cod Bay, August–September, 1932.

Station No.	Depth of mud	Bacteria per gram of dry mud						Per cent organic carbon in dry mud		
		August 24			September 21			September 21		
		Surface	2.5 cm.	10 cm.	Surface	2.5 cm.	10 cm.	Surface	2.5 cm.	10 cm.
1		43,600	9,560	3,200	—	—	—	—	—	—
2		76,000	9,680	2,000	—	—	—	—	—	—
3		217,000	11,000	—	—	—	—	—	—	—
4		236,500	16,000	7,200	2,700,000	32,200	28,800	4.50	1.77	1.90
5		134,600	24,300	9,500	1,160,000	54,700	47,700	7.50	2.46	2.85
6		248,000	44,300	15,500	8,420,000	79,300	60,000	20.00	3.10	2.76

content of the lower layers of mud is less marked. In Cape Cod Bay it was parallel with that of the surface mud, while off Cape Cod Light it was in the opposite direction. In the samples from the latter line there was again a progressive diminution in numbers of bacteria in the surface mud with distance from land. The explanation of this is not clear from the data available. In these samples the number of bacteria in the deeper layers did not decrease with distance from land. The vertical and horizontal distribution of bacteria in the mud of the Cape Cod Light line of stations is illustrated in Fig. 2. Because of the extremely wide range in the number of bacteria found, the logarithms of the numbers of bacteria are plotted, instead of the actual numbers themselves.

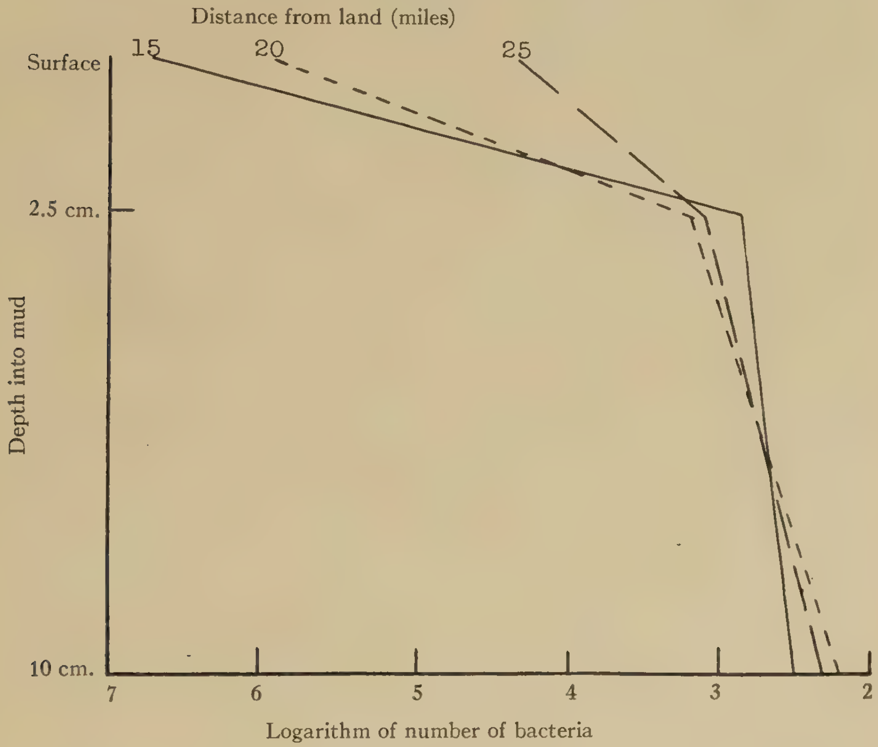

Fig. 2. Vertical and horizontal distribution of bacteria in the mud of the Atlantic Ocean off Cape Cod Light, August 24, 1932. —— Station 6; - - - Station 7; — — Station 8.

The relation between the bacterial content and the carbon content in the muds is very interesting. In the surface mud there does not appear to be a definite relation between the bacterial numbers and the amount of carbon. This is to be expected, since the number of bacteria in this layer is not only due to the abundance of organic matter, but perhaps to a greater extent to its relatively undecomposed nature as compared to that in the lower layers of mud. This dependence of the organisms upon the nature of the organic matter is illustrated also by the vertical distribution of bacteria and carbon; the number of bacteria invariably decreased with successive increases in depth in the mud, while the carbon content often rose slightly. Oceanic bottom deposits are built up principally by the accumulation of new material at the surface. The rate of deposition is extremely slow and consequently mud at relatively short depths into the mud represents sediments laid down many years before. The organic matter in the mud below the surface has undergone extensive decomposition and only the more resistant substances remain. These substances are probably similar to the resistant organic complexes in the soil described by Waksman and Iyer (1932) and are higher in carbon than is the fresh organic matter. These factors, together with the practical absence of oxygen from the mud (Moore, 1931), offer an adequate explanation for the decrease of bacterial numbers with depth in the mud. The resistant nature of the organic matter limits the number of specific organisms capable of attacking it. The high carbon and low oxygen content of the organic matter decrease its availability to anaerobic bacteria, while the absence of oxygen in the mud limits the activity of aerobic forms. At depths of 2.5 and 10 cm. in the mud the horizontal distribution of bacteria varied directly with the carbon content of the mud. This was especially true of the mud in Cape Cod Bay, where the variation in the two factors was so large as to lend greater weight to the observations. This would seem to indicate that in the terrigenous deposits examined the organic matter in the mud below the surface layer was equally available to the bacteria at all the stations sampled and probably was similar in composition. The factors determining the distribution of bacteria at the surface of the mud are still obscure. Undoubtedly an important one is the stage of decomposition of the organic matter at the time it reaches the bottom.

SUMMARY

A study has been made of the number of bacteria to be found in ocean waters and muds in the region of Cape Cod, Massachusetts, and their variation with distance from shore, depth of water, depth in the mud, and carbon content of the mud.

The number of bacteria in ocean water not near land and of a depth sufficient to preclude the agitation of the bottom by currents or wave

motion was very small. One cubic centimeter portions of the water were often found to contain no bacteria capable of growing on an agar plate. A few samples of water were found to contain comparatively large numbers of bacteria, probably due to the presence of decomposing remains of some small plankton organism.

Sea water in shallow regions where the water was mixed from top to bottom, due to tidal or wave motions, had a consistently higher bacterial content, ranging from 20 to 200 cells per cubic centimeter. Such water had a higher content of mineral nutrients and consequently a richer plankton content. It is to the larger amount of organic matter thus made available to the bacteria together with an actual transport upward of cells by vertical currents that their increased abundance under these conditions is to be attributed.

On open, exposed shores receiving little land drainage, the direct effect of the land on the bacterial content of the sea water appears to be negligible. Along shores receiving considerable land drainage any effect of the proximity of land does not appear to extend beyond a mile from the shore so far as the number of bacteria in the sea is concerned. In harbors receiving sewage drainage, the number of bacteria may rise to very high levels, depending upon the amount of pollution introduced and the degree to which circulation may take place between the harbor water and the outer uncontaminated water.

Bacteria in the bottom deposits were much more numerous than in the overlying water. Muds contained many more bacteria than did sands. The number of bacteria decreased with depth into the mud, the most marked decrease being in the first 2.5 centimeters. Bacterial numbers in the surface layer, compared to those in the lower layers, were much greater than could be accounted for by the difference in organic carbon content. There appeared to be on the surface of the mud a very thin layer containing organic matter in a less advanced stage of decomposition supporting a bacterial growth much richer not only in numbers but also in types of bacteria than in the lower layers. The carbon content of the surface layer was higher than that of the lower layers. The vertical distribution of bacteria in the mud was not related to the carbon content, since the amount of carbon sometimes increased with depth, while the number of bacteria always decreased with depth.

The horizontal distribution of bacteria in the extreme surface layer of mud was independent of its carbon content, but probably depended upon its composition or the degree of its decomposition. The horizontal distribution of bacteria in the mud layers below the surface was directly related to their carbon content.

The number of bacteria in the surface mud on the continental shelf east of Cape Cod decreased regularly with distance from land. This was not the case with the bacteria in the deeper layers of mud, where the number remained fairly constant.

There was a wide variation in the bacterial content of all layers of mud between two sampling dates approximately one month apart. The fluctuations at the surface and at depths of 2.5 and 10 cm. in the mud were in opposite directions in mud from the open ocean off Cape Cod Light but were parallel in mud from Cape Cod Bay.

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Contribution No. 20

THE WOOD'S HOLE OCEANOGRAPHIC INSTITUTION

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THE WOOD'S HOLE OCEANOGRAPHIC INSTITUTION: Sonic Soundings, and an account of a Winter Cruise to the Somers Islands[1]

JOHN COLMAN

THE Wood's Hole Oceanographic Institution is an independent organization with its laboratory at Wood's Hole, Massachusetts, where there are also the Marine Biological Laboratory and a branch of the United States Bureau of Fisheries. The first of these has an excellent biological and oceanographical library, and by friendly co-operation this is made freely available to workers in both of the other laboratories. The Oceanographic Institution is the youngest member of the group, for it was founded in 1930 and started work in 1931 under the direction of Professor H. B. Bigelow, the finances coming from the Rockefeller Foundation. The headquarters of the Institution are not the laboratory buildings, but the research vessel *Atlantis*, built by Messrs. Burmeister and Wain at Copenhagen and taken over in June 1931.

The *Atlantis* is a steel, auxiliary, Bermudian-rigged ketch, 142 feet overall, with a maximum draught of 17 feet and a displacement of 460 tons. Her masts are wooden and hollow, the mainmast rising 130 feet from the deck and the mizzenmast about 100 feet. She is double ended, with no bowsprit, and she carries a jib, fore-staysail, mainsail, and mizzen, giving a total of 7200 square feet of canvas. She will sail well within five points of the wind, which is not bad for a vessel of her size and sail area, and built primarily for work; she is stiff enough, steers easily, and her 280-h.p. Diesel engine will push her along at 8 knots in smooth water.

Just abaft the engine-room is the main winch, on whose drum are wound 30,000 feet of ½-inch diameter wire, which has a breaking strain of 12 tons and is still as good as new. This is led from a short boom by the starboard main rigging, and has been used so far for heavy plankton and trawling work, dredging not having yet been attempted. On the starboard side of the mizzenmast is the hydrographic winch, which is used for all light gear, such as water-sampling bottles, bottom samplers, small plankton nets, and so on, and also for hoisting the mainsail and the mizzen. On the deck on the port side, amidships, there is a nest of three Nova Scotia dories, so that all work has to be done on the starboard side, where the deck is uninterrupted between the main and mizzen rigging.

After the vessel was taken over in Copenhagen, she started on a six weeks' crossing of the Atlantic from Plymouth to Wood's Hole, Massachusetts. After proceeding to a point to the westward of Ireland, the *Atlantis* sailed due south till she made the Azores, and then worked west and north to her destination. During the whole of this voyage stations were worked for chemical and physical observations on the sea water, samples being taken at many depths down to 2000, and in some cases 3000, metres. These observations can be added to those made by the *Michael Sars*, the *Dana*, and other research ships working in recent years in the North Atlantic, but owing to the lapse of time between the work of different expeditions they must still be treated empirically. On this cruise the vertical distribution of plankton in relation to light intensity was also studied, and this constitutes what is perhaps the most accurate field-work on pelagic macro-plankton yet attempted.

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[1] Contribution No. 20 from the Wood's Hole Oceanographic Institution.

Since reaching America, the *Atlantis* has been based at Wood's Hole, and has made several short trips to the Gulf of Maine. In these excursions the usual water-sample and plankton investigations have been carried out, but to them has been added work on bottom-samples and their associated bacteria which may be of great importance in connection with the nitrogen cycle of the ocean and the disposal of waste and dead matter in the sea. Perhaps the happiest side of this work lies in its being undertaken by geologists, palaeontologists, and soil-bacteriologists, who are bringing in ideas and methods which might otherwise never reach the heads of people with only marine experience.

In the spring of 1932 the *Atlantis* made a prolonged cruise through the central part of the North Atlantic, including a section running due south from a point on the thirty-fifth parallel to the mouth of the Amazon on the Equator. During this voyage she was out of sight of land for seven weeks without unduly straining her fuel, food, or water supplies. In the second quarter of 1933 she has spent three months in the Caribbean Sea. Work has also been done in collaboration with the United States Bureau of Fisheries, but the central part of the first two-years' programme has been an intensive study of the water between the United States and Bermuda. This is being carried out in a series of eight quarterly cruises to that island, of which the fifth, completed in December 1932, was fairly representative of the work of the Institution as a whole, and is described later.

From the first it had been intended to equip the *Atlantis* with sonic sounding apparatus, but for financial reasons it was not until the latter part of 1932 that a "fathometer" was installed. This instrument has been fully described in the *Hydrographic Review*,[1] and the following account is based on that description. It is of the sonic, as opposed to the super-sonic, type, and the depth indications are obtained from one or other of two concentric dials; one gives depths down to 125 fathoms, the other from this depth down to about 3000 fathoms, or even more under favourable conditions. In the shoal range the sound producer is a Type 399 oscillator mounted inside the ship either in contact with the hull or suspended in a water-tank. The sound produced by this oscillator is powerful but of very short duration, resembling a heavy hammer blow. About twenty-four signals are produced per minute. The echo, after being received by two hydrophones, is converted into an electrical impulse which flashes a rotating neon tube, thereby causing a visual indication of depth to appear on the dial.

In the deep range the sound producer is a Type 324 oscillator mounted in a heavy casting which is riveted to the outside of the hull. This oscillator produces a clear musical note of about one-tenth of a second duration. Normally about twenty-four signals are produced per minute. In receiving the sound echoes from the sea bottom a tuned hydrophone is used. It is particularly sensitive to the musical note emitted by the oscillator. While soundings in the deep range are being made, a narrow beam of white light from an incandescent lamp moves steadily round a calibrated dial. The operator obtains depth measurements by observing the position of the beam of white light at the instant the echo signal is heard in the telephone receivers.

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[1] "Echo Sounding, 10, 2. Fathometer of the Submarine Signal Corporation, Universal Type 432," *Hydrographic Rev.*, 8, 2, pp. 176–183.

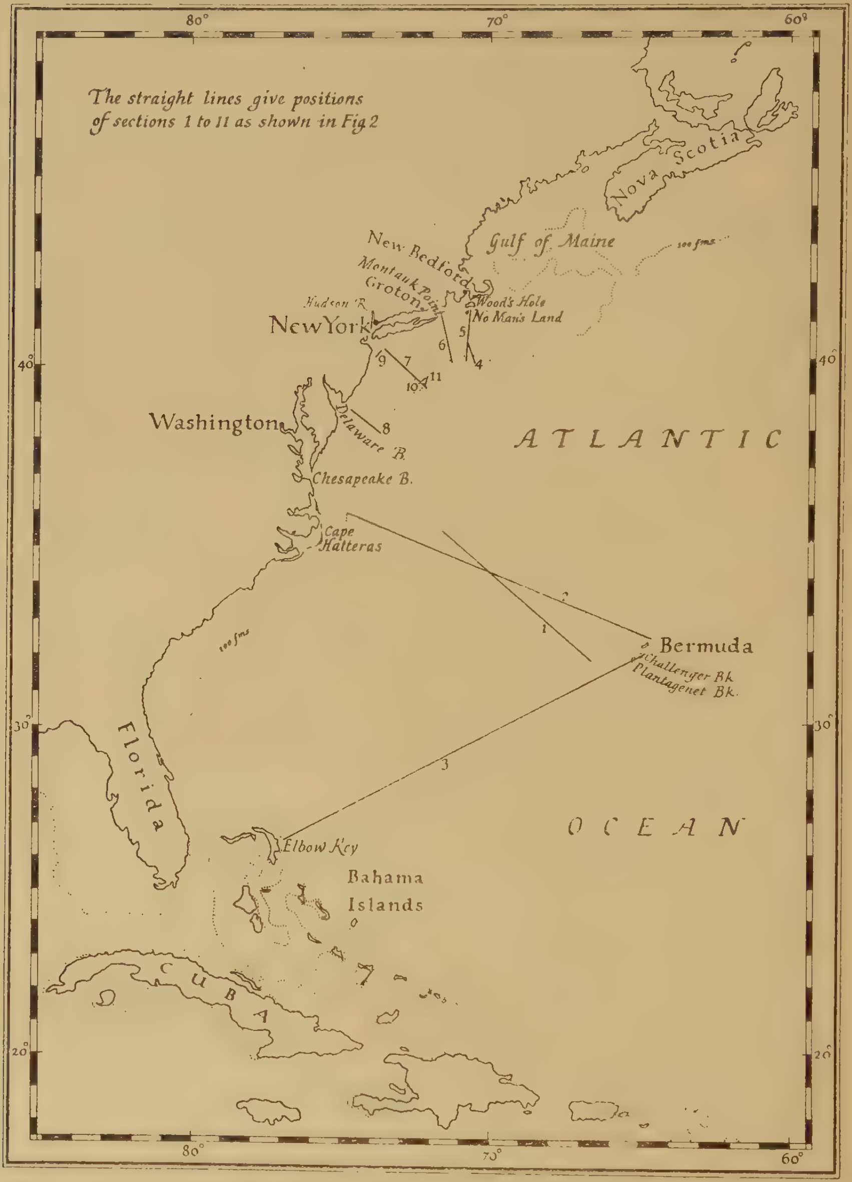
80°
70°
60°
40°
30°
20°
The straight lines give positions of sections 1 to 11 as shown in Fig 2
Nova Scotia
Gulf of Maine
100 fms
New Bedford
Montauk Point
Groton
Hudson R.
New York
Wood's Hole
No Man's Land
1
2
3
4
5
6
7
8
9
10
11
Washington
Delaware B.
Chesapeake B.
ATLANTIC
Cape Halteras
100 fms
Bermuda
Challenger Bk.
Plantagenet Bk.
Florida
OCEAN
Elbow Key
Bahama Islands
CUBA

Fig. 1. Area off the East Coast of North America in which the sonic soundings were taken

Echo sounding has now become a commonplace aid to navigation when on soundings, but it is also an almost essential part of the equipment of any vessel undertaking deep-sea oceanography, unless one is prepared to run the risk of losing apparatus through its striking an uncharted submarine mountain. In the shallow water over the Continental Shelf this is not likely, except for the possibility of wrecks on the bottom, such as one we encountered off New York, mentioned below, since it is easy to take a cast with the lead, and besides there are now very few coastal regions that are not tolerably well charted, whereas in the deeper parts of the oceans, especially away from steamer tracks, soundings may be several hundred miles apart. In shallow water also, where only a few sampling bottles are on the wire and only a limited amount of wire is over the side, the lead makes up most of the total weight. If it should strike the bottom unexpectedly, so much strain will be taken off that the winch will stop, or at any rate suddenly slow down, and give warning to those on deck. It may be mentioned that it is common practice to lower the lead into the water before attaching any bottles to the wire; this prevents the lead from swinging to the roll of the ship and so hitting the hull, and it also means that there are some 10 feet of wire below the lowest bottle, which ensures that the gear will not immediately hit the same obstruction as the lead.

In deep water however this precaution is useless, since for the sake of strength and safety fairly heavy steel wire must be employed, and several thousand feet of it weigh considerably more than the lead at the end; when to this are added the combined weights of ten or more Nansen bottles, it will be seen that should the lead strike the bottom it will make no great difference to the total strain on the winch, and that the weight of the wire and bottles will make it go on paying out with no hint on deck that anything is amiss.

This is not mere theorising, and uncharted submarine mountains do exist, even in such relatively well-explored waters as those of the North Atlantic. On 29 February 1932 the *Atlantis* was making a hydrographic station in 30° 20′ N., 51° 45′ W. This was before the installation of the fathometer, and no sounding was taken; it would have occupied several valuable hours, and anyway there were two soundings on the chart within 100 miles of the position of 2875 and 2600 fathoms, which made it appear safe enough to let down bottles at any rate to 1500 fathoms. A bottle which had reached 1713 metres or 940 fathoms came up in good order, but the deepest bottle, from 2570 metres or 1400 fathoms, was smashed, and the lead was deeply scored and had evidently been dragged for some distance over a rocky bottom. In other words, the depth was between 940 and 1400 fathoms, and was at least a mile shallower than was indicated by the chart (British Admiralty Chart 2059, North Atlantic), in spite of the fact that the printed soundings were relatively frequent.

Since the installation of the fathometer it has become part of the ship's routine to take a sounding every hour when in deep water. This unfortunately necessitates the stopping of the main engine and generator, should these be running, on account of the size of the hull. In a big ship the hydrophone which receives the echo from the bottom can be placed hundreds of feet away from the engines, the principal source of noise; in a vessel of the size of the *Atlantis* this is of course impossible, and at depths of over 300 fathoms any engine noise will drown out the sound of the echo. In still smaller ships, the noises made by

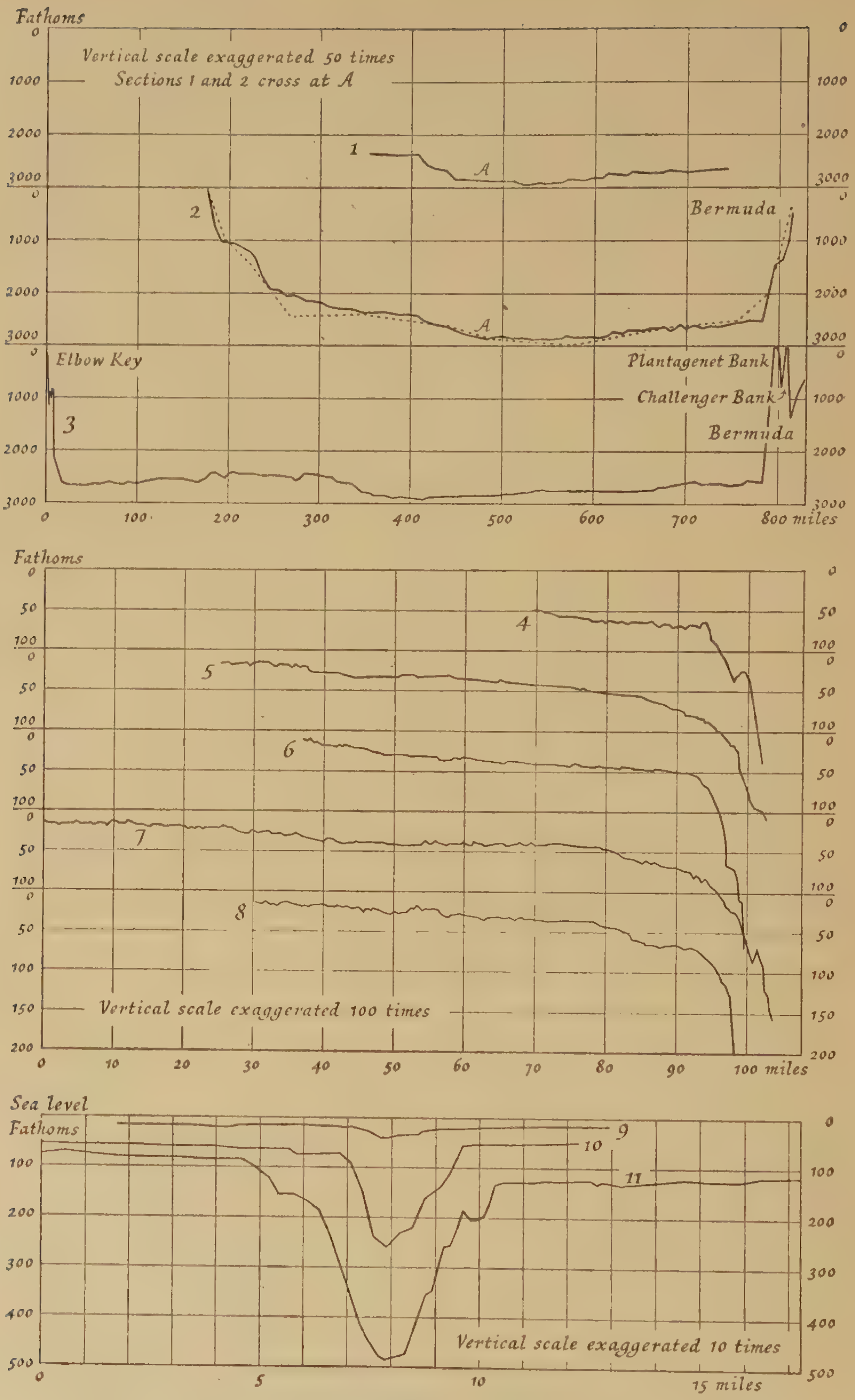

Fig. 2. Sections of sonic soundings: positions shown on Fig. 1

the hull itself become more obvious in the head-phones, so that it is probably little use to install this type of sonic sounding apparatus for deep-water work in vessels of less than 300 tons or thereabouts.

These soundings have resulted in sections 2 and 3 (Fig. 2), one from off Cape Hatteras to Bermuda, and the other from Bermuda across Challenger and Plantagenet Banks to Elbow Key in the Bahamas. These both emphasize the much more gradual slope off a continent than off an oceanic island (the Bahamas as a group being considered as the latter, since they are separated from the mainland by deeper water than that over a continental shelf). They also show what looks like a secondary shelf about 300 miles from the continent, where the depth alters in about 50 miles from 2400 or 2500 fathoms to 2900 fathoms. This outer shelf is quite steep in places, as shown by section 1, which was made on the return trip from Bermuda, but which could not be accurately drawn in as far as the coast, as for several days there were neither sun nor stars with which to fix our position.

The broken line in section 2 is derived from the soundings printed in U.S. Hydrographic Office Chart No. 1411, Halifax to Haiti, and agrees fairly well with the section made with sonic soundings except in the entire absence of detail, and in the case of one sounding about 80 miles off the Shelf. Here the fathometer gave 2050 fathoms, but the chart 2450 fathoms, a difference of nearly half a mile. This position is in the path of the Gulf Stream, and it seems probable that the sounding in question was taken by the lead, in which case the relatively rapid surface drift would have taken the ship away from a position vertically above the lead; this factor could easily introduce errors of this magnitude. It would be a welcome innovation if, in future editions of oceanic charts, echo soundings of more than 500 fathoms were to be distinguished typographically from soundings made with the lead. The latter are rarely accurate, and may be very wrong, and can only be taken as indications of depth.

In Fig. 2 are drawn out five sounding sections across the brow of the Shelf, so arranged on the figure that the 100-fathom lines are vertically above one another. In three of them, Nos. 5, 7, and 8, the pitch of the slope increases appreciably at about 40 or 50 fathoms, but does not become really steep until 70 or 80 fathoms, when it plunges into the abyssal depths; in Nos. 7 and 8 this double break is so marked that the sections appear concave. Sections 4 and 6 resemble each other in having a very sharp edge to the shelf, but although they are quite close together, only 40 miles apart in fact, section 5, which is between them, shows a very different profile, with no sharp edge. It would seem that here is a geological problem, concerned with the nature of the rock underlying the bottom. The sea-floor appears to be more uneven in the more southerly sections, but it must be kept in mind that the vertical scale is 100 times the horizontal, so that the corrugations are actually very slight.

Finally, there are three sections across the Hudson River Mud-Gorge, shown in Fig. 2 on a vertical-horizontal scale of 10 to 1. Similar deep submarine valleys are quite common, and have been recently discussed in the *Geographical Review*.[1] They are very definite in shape and, for submarine slopes, of astonishing steepness. The form is usually that of a V, the rims are very sharp, and the actual dimensions are comparable with those of the biggest terrestrial valleys. For instance, the Grand Canyon of the Colorado River, in Arizona, among the most spectacular chasms in existence, is about 1 mile deep, and 13 miles wide at the narrowest part; the Hudson Gorge, at section 11, is about 400 fathoms, or a little less than half a land mile, deep below the brim, and 5 sea miles wide. This is deeper in proportion than the Grand Canyon, in spite of the sediment that one would expect to be accumulating in the Mud-Gorge. These valleys extend seawards as clefts in the Continental Slope to depths of more than 1000 fathoms; good examples are those off the Congo, off the Ganges, and in Monterey Bay, California, where there is at present no sizeable river. The whole question of their origin and maintenance is a geographical and geological problem of the greatest interest.

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[1] Francis P. Shephard, "Submarine Valleys," *Geogr. Rev.*, 23, 1, January 1933.

On the cruise during which most of these soundings were made, we left Groton, Connecticut, on the Thames River, at 7.30 a.m. on 27 November 1932, and after rounding Montauk Point, sailed south by west. It was blowing a force 7 breeze from north by west and was very cold, with 20° of frost. The sea-water however was at 40° F., and the difference in temperature caused the formation of flecks of mist on the surface of the water; they looked just like balls of cotton wool rolling along at 20 miles an hour. They disappeared about 9 o'clock, when the sun was properly up. Soon the wind veered till we were running dead before it, and for more than thirty hours we averaged 8 knots under the main trisail only; the headsails were blanketed and of no use, and the mizzen could not be hoisted as it made the ship gripe too heavily. This fine sail was marred only by our rolling steadily 20° to windward and 25° to leeward all the way to Cape Hatteras. On the way we crossed the Hudson Mud-Gorge, making section 11; the soundings ran from 82 fathoms to 482 fathoms and back to 130 fathoms, all in 5 miles. The gorge has been used for centuries by vessels making New York on the lead in foggy weather.

It had been our intention to start a line of hydrographic stations from the shallow water at the mouth of Chesapeake Bay, out to the deep sea and on to Bermuda, but when we got to the Continental Shelf to the northward of Cape Hatteras it was still blowing far too hard to justify an approach to a lee shore, or to let us do any work, so we hove to for the night. The following day the wind had eased to force 5, and we sailed for the Shelf; although we were reaching under the somewhat reduced rig of jib, main trisail and mizzen (the staysail having been punched full of holes by the jib-sheet blocks), we averaged 10·2 knots for eight hours, a grand piece of sailing. After working our first station in 80 fathoms, we laid a course for Bermuda, and at last everything went smoothly. Stations were at first 15 miles apart, until we were across the Gulf Stream, then spaced out to 20, then to 30, and finally to 60 miles in the very constant and uniform waters between the Stream and Bermuda. At all stations, except those on the Shelf, samples were taken at sixteen depths down to 1200 metres, and sometimes deeper still, for temperature, salinity, and for phosphate content; some samples were also collected for nitrate and for diatoms.

The apparatus used was the Nansen bottle, which still more than holds its own as the best collector of water samples. It is one of the very few pieces of marine gear which merit the title of instruments of precision, but in spite of its great accuracy it will stand a surprising amount of rough treatment. The thermometers (two per bottle) are of the reversing type, the mercury thread being broken before the bottle is brought to the surface, and they read accurately to 1/100° C. The bulb of one thermometer is surrounded by an outer glass case containing air, which prevents the pressure of the water affecting it, but the bulb of the other thermometer is in direct contact with the water, and will read about 1° C. too high for each 100 metres of depth. Comparison of the two readings will, after a simple calculation, give the precise depth from which the sample has been taken. This is an essential procedure, since except in the very rare combination of a flat calm and no current there is always a certain amount of drift going on, with a consequent departure of the wire from the vertical; in shallow-water work this can be allowed for after observing the angle between the wire and the vertical or horizontal, but in deeper sampling there is no certainty or even likelihood that the wire follows a regular path through the water to the lowest bottle, so that the use of unprotected thermometers is the only practical and reliable method for ascertaining the depths to which the bottles have actually been sunk.

On the four previous Bermuda trips, the hydrography had given almost identical results, so that it was beginning to be thought that this part of the sea was becoming fairly well known. The isotherms, which are crowded together over the Continental Shelf (where the bottom waters, though shallow, are always rather cold), drop precipitously at the north-western margin of the Gulf Stream, and then remain deep and widely spaced through the central part of the North Atlantic south-east of the Stream. In other words, the Stream is not so much a sort of river flowing over the surface of the ocean, an idea put forward in not a few geography books, as a vertical wall of considerable depth, having on its left hand cold, coastal water, but on its right hand warm, oceanic water much more like its own in composition. On this fifth trip however the isotherms all rose again on the south-eastern side of the Stream before returning to the levels which they maintain with little variation to beyond Bermuda. This, in conjunction with other data, is interpreted as an indication of a strong movement of water parallel but opposite to the Gulf Stream, perhaps part of a big vortex. All depths down to 2000 metres were affected, including the surface, as indeed we found out at the time when we were 50 miles beyond the Stream, for the *Atlantis* was set for a while to the southward of her dead-reckoning position.

From here we rolled on to Bermuda in beautiful weather, but with a big northerly swell and not enough wind to fill the sails. It had been our plan to stop only two days at the island, to get rested, but as our next job needed a calm sea and a light breeze, it was not surprising that we were kept for five days in St. George's Harbour until a very fresh north-easterly breeze died down. We then left Bermuda and sailed south for a day, and for two days had just the right conditions, except for the eternal and inconvenient swell. This calm-weather work was in connection with the retarding effect of the sea's surface on the wind, or, alternatively, with the propelling effect of the wind on the surface layers of the sea, and was bound up with the theoretical path, known as the Ekman Spiral, of a fluid mass moving over the surface of a rotating sphere.

On a previous trip the effect of the sea on the wind had been studied by releasing pilot balloons and plotting their paths through the air by taking azimuth readings and measuring their height trigonometrically at given intervals of time. This proved too difficult from the deck of a small and not unlively ship to give much beyond qualitative results; the method is however being persevered with. The experiment on our trip concerned the other aspect of the problem, namely the effect of the wind on the sea, and it consisted in plotting the paths of glass balls ascending through the water at known rates from known depths, the balls being such as are used by fishermen as floats for their drift nets. Each ball was attached to a metal cone which kept its ascension rate down to about a foot a second, and they could be attached in a series down the wire and released by a messenger in much the same way as a series of Nansen bottles.

Let us suppose that the deepest ball will be released at 50 fathoms, with others on the wire between that depth and the surface. Then, if the ship and all the water down to that depth are stationary, or drifting in the same direction and at the same speed, the released balls will all rise apparently vertically, and come to the surface together close to the wire. If there is any differential drift, the balls will come up away from the ship at varying distances and directions, which can be measured by means of range-finder and azimuth readings. In practice it was found difficult to use a range-finder at all accurately when the balls were being frequently hidden by swells; the mizzen rigging or the wheel-house kept on getting into the line of vision of the man taking compass bearings, and here again only qualitative results could be obtained. An additional complication must be the turbulence caused by a rolling vessel drawing 17 feet; this turbulence could be plainly felt while swimming under water 30 feet from the ship. I believe, though, that this work could be done, with interesting and perhaps important results, by lowering and releasing the balls from a large raft, fitted with a 10-foot tower from which to take compass bearings and range-finder readings.

Having done what we could with the balls, we now set a course for the mouth of Delaware Bay, and every night took tows for plankton with two tow-nets each 6 feet across at the mouth. The main objective was the collection of eel-larvae for the late Professor Schmidt, of Copenhagen, who worked on these larvae from all over the world for a matter of thirty years, but we also experimented with a new net in an attempt to make collections of really deep plankton which should be uncontaminated by the richer material from the upper layers. For this purpose we tried out a net which theoretically could be sent down closed to the required depth, opened with a 5-lb. messenger, towed for a given length of time, and then closed again with a 20-lb. messenger, before being brought to the surface. A small net of such design has been used very successfully, but since the plankton of deep off-shore water is exceedingly scarce, we had to use much bigger and more cumbersome gear. It consisted of the usual type of conical net, and a special frame which was 8 feet wide at the mouth and weighed upwards of 200 lb. It was attached to the release mechanism, which was of heavy brass construction, by some four or five lengths of wire, varying from 10 to 40 feet. In designing it all on paper it was simple to draw the wire nice and straight and make it look easy to work, but in practice there is nothing quite so perverse as flexible steel wire, and usually one or two of the bridles threw a half-hitch round some part of the net or frame and prevented it from working as it should. Moreover we were working at night, since we combined these deep tows with those for eel-larvae; it is no use fishing for the latter by daylight, as apparently they can then see the net coming and swim out of its way.

There was no fault to be found with the apparatus theoretically, and we struggled with it on several successive nights, but finally, after the gear had gone over the side looking really unentangled for the first time, it came up with the net torn completely away from the canvas band connecting it with the frame; this band, which had been bent to the frame with some thirty turns of heavy serving wire, was left attached by two turns only, and the frame itself was buckled, though made of galvanised iron half an inch thick and 1½ inches wide! Either it was not strong enough to withstand the sudden shock of the net opening and filling with water, or else we had caught, and lost, a monstrous fish. All hands were profoundly grateful when this piece of gear was finished with; it was certainly too big and clumsy to be used on a vessel with the deck arrangements of the *Atlantis*, some sort of outboard derrick being essential.

Our arrival at the Continental Shelf off the Virginia Capes neatly coincided with that of a whole gale from the north-east. The barometer, which had been rather high, dropped almost an inch in four hours, and the wind blew hard for twelve, after first ruining the jib. Then it eased for half an hour, but came in at storm force from the north-west while the glass busily climbed back again; at no point had it been below 29·3 inches, and the whole affair was over in twenty-four hours. It was of short duration compared with a typical winter gale on the European side of the North Atlantic, but it left quite as disturbed a sea. Fortunately however our programme now took us in towards the land where we were fairly well protected. We made three more hydrographic sections across the Shelf in very fair comfort, though to be sure it was cold sailing close-hauled up the New Jersey coast; it was a luxury to have no swell.

The first of these sections was from the edge of the Shelf into the mouth of Delaware Bay, the second out from near the Ambrose Lightship off New York, and the last one in from south of No Man's Land until we made Gay Head Light as our landfall. On these runs the Nansen bottles again worked perfectly, as is their custom, and indeed on the whole the cruise, during which we collected more than four hundred water samples, not a single bottle failed to do its work, except on three occasions which were due to our own negligence, and when it was a simple matter to repeat operations. While on the shallow waters of the Shelf we were also taking sonic soundings every three minutes, partly out of curiosity to see if the bottom were really as smooth as the soundings on the chart led one to expect, and also to get detailed profiles of the brow of the Continental Slope. The fathometer helped us most materially in another direction however when we arrived at the position of one of our stations, at 39° 49′ N. by 72° 54′ W. For some time the echo soundings had been agreeing with those on the chart at 40 or 41 fathoms, but as we hove to we suddenly got only 32 fathoms. We checked and confirmed this figure with the lead, and for a while got various readings from 32 to 41 fathoms before drifting clear; it was presumably a wreck, and a big one too, to judge by the difference between the extreme readings. A single cast with the lead, if it had agreed with the chart, would have given us confidence to let down our bottles to within 2 or 3 fathoms of the bottom, and this might well have tangled them up in the wreck or whatever the obstruction was.

On the last section in towards Gay Head our latitude was somewhat uncertain owing to our not having had a good afternoon sight, and the captain wanted to know exactly when we crossed the 50-fathom line, which hereabouts runs almost due east and west. With the fathometer this was simplicity itself, while the use of the lead would have entailed the fuss and bother of rounding to every so often in the fresh breeze that was then blowing. Finally, we arrived at New Bedford for Customs and Quarantine early on the morning of December 23, and reached Wood's Hole late the same evening.

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LIGHT INTENSITY AND THE EXTENT OF ACTIVITY OF LOCOMOTOR MUSCLES AS OPPOSED TO CILIA

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I

The effect of light on animal behavior has attracted the attention of biologists for many years. One phase of the general problem, namely the photokinetic effect of light, has been studied to a less extent than orientation and the factors determining the phototropic condition of organisms and many questions regarding this phase of photic behavior remain to be answered. The first and most important of these is whether or not intensity of illumination does actually influence the speed at which an animal moves toward or away from a source of illumination. Some of the evidence has already been reviewed by the author (Welsh, 1932*a*). In the same paper and in additional work it has been shown that in a light-sensitive organism, which moves by means of muscles, the extent of muscular activity bears a very definite relationship to intensity of illumination. This is not in agreement with work of Mast (1923), and Dolley (1917), but does agree with the results of most investigators and seems reasonable in the light of what we know regarding the response of the receptor-muscle system to light. It is not as evident that light intensity has a similar effect on organisms which move by means of cilia or flagellæ. Observations on such forms indicate that there is no effect of luminous intensity on speed of progression. Directional light, however, does influence orientation in these animals and it is difficult to determine whether slight changes in rate are due to deviations from a straight path or to changes in amplitude and frequency of ciliary movement. It seemed desirable to search for forms which move by means of cilia and which, after primary orientation, proceed in a straight line. A study of such forms should indicate whether or not their speed of progression is a function of the intensity of illumination.

In the course of some work at the Woods Hole Oceanographic Institution there was an opportunity for such an investigation and a survey was made of the effect of light intensity on the activity of a number of marine invertebrates. The results indicate that the extent of ciliary activity is not a function of the amount of light energy reaching the organism as is true of the activity of locomotor muscles.

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[1] Contribution No. 21 from the Woods Hole Oceanographic Institution.

II

Among the animals used in the investigation were two species of small marine turbellarians, one of which was identified as *Plagiostomum* sp.; the other has not yet been identified. These were obtained largely from *Ulva* where they were found moving over the surface of the alga. They both orient by means of muscles but after primary orientation cilia alone seem to be concerned in locomotion. One important difference made these animals particularly valuable for purposes of comparison. *Plagiostomum* tends to be free-swimming and the path through which it moves is not straight, while the unidentified form always moved in contact with the substrate and its path was a nearly perfect straight line. *Columbella lunata,* a small gastropod, also moves by means of cilia. Orientation again involves muscles but they appear to play no part in forward locomotion. *Arenicola* larvæ were also used but the spiralling of this form made it difficult to distinguish between orientation effects and any change in extent of ciliary activity.

Among the animals observed which move by means of appendages operated by muscles were larvæ of *Pinnotheres maculatus, Hippa talpoida,* and stomatopod larvæ, probably of *Squilla empusa.* The copepod *Centropages typicus* and small specimens of *Limulus polyphemus* were also used. All of these forms, at the age used, were positive to light with the exception of *Limulus* which was negative.

The experiments were carried on under carefully controlled conditions. The animals were placed individually in a glass trough, 30 cm. in length, with the bottom marked in centimeters. This trough was partially submerged in a water bath maintained at 21.3° C. Light reached the animals through windows in opposite sides of the tank. The light sources were two ribbon filament lamps arranged to give an even illumination of nearly parallel rays in the mid-region of the trough, the illumination from one lamp being 1500 foot candles at this point and from the other 7.5 foot candles. These intensities were selected after numerous tests on different species had shown that the intensity of 1500 foot candles was higher than that necessary to elicit a maximum speed, while 7.5 foot candles fell within the range of intensities to which these same organisms showed a change in speed of progression (*e.g. Pinnotheres* larvæ, Welsh, 1932*a*; *Unionicola,* Welsh, 1932*b*).

Both lamps were operated from a three-way switch so that one was turned off as the other was turned on.

The animal under observation was adapted to the temperature of the sea water in which it was placed and then timed while traversing the middle 10 cm. of the trough. Ten trials were made for a given animal at each of the two light intensities, and the times were averaged. The results are shown in the following section.

III

For an analysis of the findings the animals may be divided into three groups according to their methods of locomotion and the extent to which orientation may affect the apparent velocity of forward progression. We may place in the first group those animals which move by means of locomotor appendages operated by muscles; in the second group animals moving by means of cilia and exhibiting considerable variation from a straight path in directional light; and in the third group animals moving by means of cilia and progressing in a straight line in directional light.

TABLE I

The effect of light intensity on the speed of forward progression of animals moving by means of locomotor appendages operated by muscles.

Animal	I = 1500 ft. candles		I = 7.5 ft. candles	
	Time for travelling 10 cm.	P. E.	Time for travelling 10 cm.	P. E.
	seconds		*seconds*	
Pinnotheres larva	6.6	±0.13	14.1	±0.47
Hippa larva	4.5	±0.06	11.1	±0.16
Squilla larva	4.9	±0.45	11.3	±0.66
Limulus	5.4	±0.05	7.4	±0.25
Centropages	4.4	±0.22	8.5	±0.77

Table I gives the average times, with their probable errors, for the first group. The results indicate that light intensity greatly influences the speed, as the times for travelling 10 cm. at the lower intensity are over twice as great as those at the higher intensity in all instances but one. This indicates that the frequency and amplitude of movement of the locomotor appendages must increase with an increase in intensity, as is true for *Unionicola* (Welsh, 1932*b*). This may be accounted for by assuming a more rapid decomposition of photo-sensitive material in the photoreceptor as shown by Hecht (1920), followed by a greater frequency of discharge over sensory tracts from the eye (Hartline and Graham, 1932), and finally more muscle fibers of the locomotor appendages being stimulated by discharge of motor nerves. Throughout this system thresholds of receptors, conductors, and effectors may be involved as well as frequency of discharge of the nerves, and it is readily apparent that the amount of light energy reaching the organism might well be expressed by the degree of activity of locomotor structures, and hence the speed at which the animal travels.

Table II gives the times for traversing 10 cm. for three different animals belonging to a species of *Plagiostomum,* and for a larva of the lug-worm *Arenicola.* In both forms cilia are involved in forward locomotion but there are considerable deviations from a straight path even in progression toward a light of high intensity. The times for travelling 10 cm. at the lower intensity are greater than those for 1500 foot candles, but in the case of *Plagiostomum* the difference is not large and there is no agreement between the average times and their probable errors as is shown in Table I. The probable error in each instance is less for the lower intensity than for the higher. It seems in the case of *Plagiostomum* that the difference in times at the two intensities might be accounted for by the deviations from a straight path which are greater at the lower of the two intensities. These deviations involve muscular bending of the body which helps in no apparent way with forward progression.

TABLE II

The effect of light intensity on the speed of forward progression of animals moving by means of cilia and exhibiting considerable variation from a straight path in directional light.

Animal	I = 1500 ft. candles		I = 7.5 ft. candles	
	Time for travelling 10 cm.	P. E.	Time for travelling 10 cm.	P. E.
	seconds		*seconds*	
Plagiostomum (1)	52.6	±2.83	61.8	±2.75
Plagiostomum (2)	33.9	±0.84	37.7	±0.65
Plagiostomum (3)	35.8	±1.80	42.6	±1.03
Arenicola larva	78.5	±1.02	123.5	± 5.6

In the case of the *Arenicola* larva the difference in times at the two intensities is proportionally greater than that for *Plagiostomum* but the deviations from a straight path were more pronounced. At the lower intensity the degree of spiralling increased and also an up and down movement was more noticeable.

Now if we examine the results from another turbellarian and from *Columbella lunata* (Table III), it is seen that intensity of illumination has no effect on the speed of progression. In these forms, as stated previously, muscles play an important part in orientation and the angle of turning bears a definite relationship to the luminous intensity. After orientation, however, the movement toward the light was a smooth, gliding one, and the deviations from a straight path were negligible. While there was considerable variation in the speed of different animals of the same species, the speed of any particular specimen was quite uniform, and this was true irrespective of light intensity.

These results would indicate that the beat of cilia is not affected by light intensity as is the frequency and amplitude of movement of locomotor muscles. These two types of effectors are quite different in their behavior to stimuli other than light, and there is no known mechanism whereby the amount of light energy reaching the photoreceptors might be realized by the cilia except in an indirect way.

TABLE III

The effect of light intensity on the speed of forward progression of animals moving by means of cilia and progressing in a straight line in directional light.

Animal	I = 1500 ft. candles		I = 7.5 ft. candles	
	Time for travelling 10 cm.	P. E.	Time for travelling 10 cm.	P. E.
	seconds		*seconds*	
Turbellarian sp. ? (1)	49.5	±0.57	50.1	±0.50
Turbellarian sp. ? (2)	89.1	±2.63	89.6	±2.48
Turbellarian sp. ? (3)	37.9	±0.28	37.4	±0.44
Columbella	80.2	±2.15	81.2	±2.42

IV

The results obtained on animals progressing by means of cilia are in agreement with those of Holmes (1903), and Mast and Gover (1922) obtained on flagellated forms, but they indicate that the laws governing these forms cannot be applied to forms such as the arthropods which move by means of appendages operated by muscles.

In the case of planaria there is an apparent effect of light on speed of progression (Parker and Burnett, 1900; Pearl, 1903; Walter, 1907), but in these animals, according to Stringer (1917), locomotion is essentially a muscular act in which cilia play no necessary part. Evidently only in the smaller turbellarians do cilia function as important locomotor structures. This is probably also true of the gastropods.

The observations of Umeda (1927), Wharton (1931), and Lucas (1933), as reported by Lucas, concerning the effect of light on ciliated epithelium, are of interest in this consideration although they are somewhat contradictory. Lucas (1933) found that in ciliated epithelium from the frog, quiescent cilia were sometimes made active by illumination. He reports in the same paper that Umeda obtained similar results from illumination with light of long wave lengths. Wharton (1931), however, in studying cilia of the tracheae, found that light had an apparent inhibitory effect on the beat of the cilia. As the present writer is not familiar with the experimental technique used by these investigators it is not possible to comment on the results. It is difficult to conceive of light, dissociated entirely from heat, having a direct effect on the activity of cilia of ciliated epithelium although such an effect may be possible. This problem would seem to be worthy of further investigation.

It seems safe to conclude from the evidence at hand that the activity of locomotor muscles of certain animals, and hence the speed of progression of these forms, is a definite function of the intensity of illumination over a given range, while the activity of cilia, and speed of animals moving by means of cilia bears no such relation to light intensity.

Summary

The results obtained from a study of the effect of intensity of illumination on the speed of progression of a number of marine invertebrates indicate that the activity of locomotor appendages, operated by muscles, is a function of the luminous intensity and that the animals move faster in light of high intensity than in light of low intensity. This, however, is not true of animals moving by means of cilia, for such animals, provided they move in a straight line in directional light, show no change in speed and hence no change in ciliary beat as the light intensity is varied.

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Contribution No. 26

OBSERVATIONS ON THE PENETRATION OF DAYLIGHT INTO MID-ATLANTIC AND COASTAL WATERS

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Although considerable information on the penetration of daylight into the sea has been obtained from measurements made by Shelford and Gail (1922), Shelford (1929), Poole and Atkins (1929), Atkins (1932), and Atkins and Poole (1933), it was desired to extend this type of investigation to other regions and to study in further detail the effect of varying conditions upon submarine irradiation.[2] It was hoped to make observations in the open ocean, where the photo-electric method had never before been employed, and to compare the intensity of irradiation found there with that existing in coastal regions. As far as possible it was desired to learn to what extent the degree of light penetration is modified by changes in latitude, in time of day, and in condition of the sea surface. This information is of particular importance in relation to the theories for the vertical distribution of plankton (cf. Russell, 1927; 1931). We therefore proposed to measure the intensity of submarine irradiation at the same time that plankton hauls were being made. The application of our results to the theory of the effect of light on the diurnal migration of plankton is considered in a separate paper (Clarke, 1933).

The observations presented in this paper were made from the "Atlantis," the research vessel of the Woods Hole Oceanographic Institution. The photometric apparatus was put aboard at Plymouth, England, in July, 1931. Measurements of the penetration of light into the sea were made at thirteen stations on the trip across the Atlantic during the succeeding six weeks. The work was continued in July and August, 1932, when several series of observations were carried out in the Gulf of Maine and in Woods Hole Harbor.

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[1] Contribution No. 26.

[2] By irradiation is meant the radiant flux density of all wave lengths (cf. Sawyer, 1933), as distinct from illlumination which refers to visible wave lengths only. The term "radiation" has formerly been used loosely for irradiation.

APPARATUS

The apparatus for the measurement of submarine irradiation assembled for the "Atlantis" has been described in detail by Gall and Atkins (1931). Each photometer contains a potassium (sensitized with hydrogen) photo-electric cell. Two of the cells are of the vacuum type; the third is gas-filled. The photo-electric current is measured with a potentiometer or neon lamp outfit. Essentially, the method consists in measuring alternately the intensity of daylight falling on deck and that penetrating to various depths beneath the surface of the sea. Values are thus obtained for the submarine irradiation as percentages of the light at the surface. Absolute values of the intensity of light are derived by calibration of the instruments against a photometric standard.

With the original arrangement of the apparatus considerable trouble was caused by the fouling of the lines intended to operate the shutter of the submarine photometer and by the horizontal straying of the instrument due to the drift of the ship and the great sideward resistance of the lines and cables. Some improvement was produced by replacing one line by a heavy weight which opened the shutter. However, fouling and straying were still troublesome in rough weather and whenever working at depths greater than 50 meters. Accordingly, the No. 1 photometer was provided with a new four-conductor insulated cable and with an internal shutter which can be opened and closed electrically.

The circuit for the internal shutter uses two of the conductors of the new cable. The other two conductors are used for the photoelectric circuit. The new cable was made for the Woods Hole Oceanographic Institution by the General Electric Company. It is 300 meters long, covered with a very tough rubber jacket, and is capable of withstanding a strain of 168 kilograms without injury. The original five lines running to the photometer from the ship have thus been reduced to two, viz., the steel supporting cable and the new four-conductor cable. Fouling never occurs even in rough weather, and since the diameter of the new cable is only 1.7 cm., the sideward resistance of the whole apparatus is greatly reduced. During the work following these improvements the supporting cable rarely reached an angle with the vertical greater than 20°.

The original external shutter has been left attached to the photometer case for protection of the glass window when not in use. Just before the photometer is swung over the side, this shutter is tied open. The internal device was made from a Packard photographic shutter, the aperture of which is 9 cm. in diameter. The rubber bulb, which ordinarily operated the shutter, has been removed and a short lever arm attached to the post which, when moved to the left or to the right, opens or closes the leaves of the shutter. The lever arm is arranged as the armature of an electro-magnet (see Fig. 1). The free end of the lever arm, which is attached to the shutter, swings to the left or to the right according to whether the left-hand pole or the right-hand pole of the magnet is activated. Each pole is wound with 500 feet of double silk insulated No. 30 copper wire. One side of each coil is grounded to the case (and hence through the sea water and supporting steel cable to the hull of the ship).

The operation of the shutter is controlled by a double throw switch conveniently located in the deck laboratory near the potentiometer. The ship's current is used to activate the coils of the electro-magnet (see Fig. 1). Closing the circuit momentarily suffices to open the shutter, or to close it. The shutter remains in the position in which it is left when the current is cut off. Since the current is allowed to flow for only a short interval, the coils may be greatly overloaded, thus increasing the strength of the magnet. Ordinarily 0.4 ampere is sufficient to operate the shutter but the current may be increased to one ampere if necessary. This method has the additional advantage that no current is flowing through the electro-magnets while measurements in the photo-electric cell are being made.

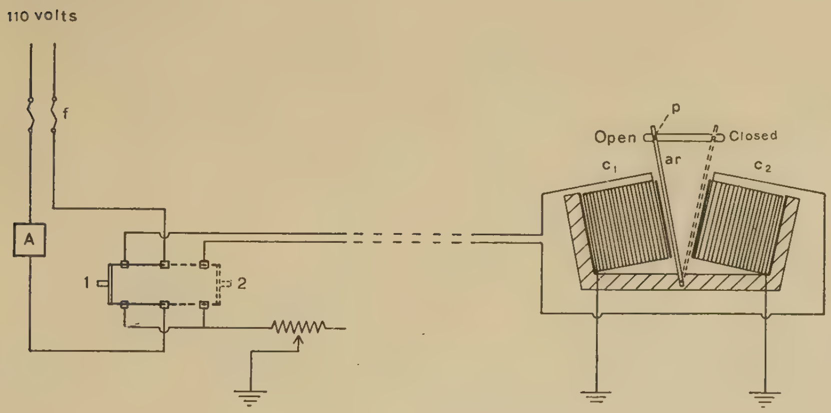

FIG. 1. Diagram of the connections of the electrically operated shutter.
(1) Position of switch to open shutter.
(2) Position of switch to close shutter. Except for the moments of operating the shutter, the switch is left with contacts broken in the vertical position.
A, ammeter; *ar*, armature; c_1, c_2, coils of electromagnet; *f*, 1-ampere fuses; *p*, post attached to leaves of shutter.

STANDARDIZATION OF PHOTOMETERS

Before the "Atlantis" sailed from Plymouth, the photometers were compared by Dr. W. R. G. Atkins with a vacuum potassium cell which had been standardized previously against an open carbon arc lamp (see Gall and Atkins, 1931, where the lowest and highest intensities of light which can be measured by our apparatus are stated also). The values obtained by him are summarized in Table I.

Birge and Juday (1932, p. 525) have pointed out that the meter-candle is not a suitable unit for the measurement of irradiation under water. This unit is misleading when applied to the visible part of the solar radiation penetrating beneath the surface because the energy spectrum has become so profoundly modified. Moreover, the radiation beyond the limits of the visible should be measured in addition, and this cannot properly be expressed in meter-candles. Whatever unit is used, the following precaution must be taken in order to obtain a correct standardization of the photometer: in evaluating the standard source only that region of its spectral emission may be considered which

TABLE I

Standardization of photometers. Irradiation necessary to produce photo-electric current of 1 microampere at an anode potential of 60 to 63 volts.

Photometer	Carbon arc used as a standard *	1000-watt Mazda lamp used as a standard		Photo-electric emission produced by test lamp (used to check for changes in sensitivity at sea)
	Irradiation extending over visible range	Irradiation extending over visible range	Irradiation within range: 3460A°–5260A°	
	meter-candles	*meter-candles*	*microwatts/cm.^{2}*†	
Deck	370	1610	135	4.4 μa
No. 1 Sea (1931)	870	2080	175	—
No. 1 Sea (1932)	—	3760	315	2.1
No. 2 Sea	232 ‡	750 ‡	63 ‡	—

* Values given by Gall and Atkins (1931).

† 1 Microwatt/cm.^{2} = 10 ergs/cm.^{2}/sec. = .0000143 gm. cal./cm.^{2}/min.

‡ Values obtained immediately after glowing the cell for one second (see Gall and Atkins, 1931).

It will be observed that the ratios of the carbon arc and the Mazda lamp standardizations are not the same for the three photometers. Evidently the sensitivities of the photo-electric cells changed considerably during the year which elapsed between the tests.

The decreased sensitivity of No. 1 as used in 1932 was caused by the greater distance between the photo-electric cell and the photometer window. This change in position was necessary in order to make room for the new internal shutter.

occurs within the spectral range to which the photometer actually used is sensitive. Measurements made without this precaution give an erroneous idea of the submarine irradiation and the actual values obtained depend upon the type of source used for standardization. An illustration of this is to be found in Table I, where three standardizations of our photometers are compared. The values in meter-candles based upon the carbon arc differ widely from those based upon the incandescent lamp. The discrepancy is due to the fact that the whole visible radiation of the two standards has been measured and that these differ in spectral distribution, particularly in the region to which our photometers are most sensitive (see below).

Submarine irradiation may be properly expressed in cal./cm.^{2}/min., in ergs/cm.^{2}/sec., or in microwatts/cm.^{2}, provided that the spectral range measured is stated. In this paper calibration values are given in microwatts/cm.^{2} for the narrow range within which our photometers are sensitive. To facilitate comparisons between different investigations it is desirable to state the irradiation at any depth in the sea as a percentage of that just over the surface, the measurements being made in the same way. The values may then be ascertained readily in terms of any unit which was found convenient for the determination of the irradiation in air. This method is used in the present paper. The transmissive exponent,[3] k ("absorption coefficient"), can be readily derived from the percentage data by applying the usual formula:

$$\frac{I}{I_0} = e^{-kL}$$

where $L =$ thickness of water layer (expressed in meters) in which the intensity is reduced from I_0 to I. Convenient tables for the calculation involved are provided by Sawyer (1931).

The spectral sensitivities of our photo-electric cells (without the cases, but with the diffusing discs in position) have been determined by the U. S. Bureau of Standards. The curves are reproduced in Fig. 2, where it will be seen that no significant differences exist. For purposes of standardization the range of sensitivity of the photometers was not considered to extend to the extreme limits of the curves, for to do so would result in the inclusion of a relatively large amount of the standard lamp's radiation in the yellow which would be responsible for a very small part of the photo-electric current. The limits of the range of sensitivity of the photometers were arbitrarily set at 3460A° and 5260A° respectively. This range extends 900A° above and below the point of average maximum sensitivity (4360A°) and includes over 96 per cent of the total area of each curve.

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[3] It is the *vertical* transmissive exponent that is actually measured. The question of the direction of light rays beneath the surface has been discussed by Atkins (1932, p. 184).

The standardization of each photometer was carried out against a 1000-watt Mazda " C " lamp, manufactured, seasoned, and rated by the General Electric Company. The data supplied give the irradiation

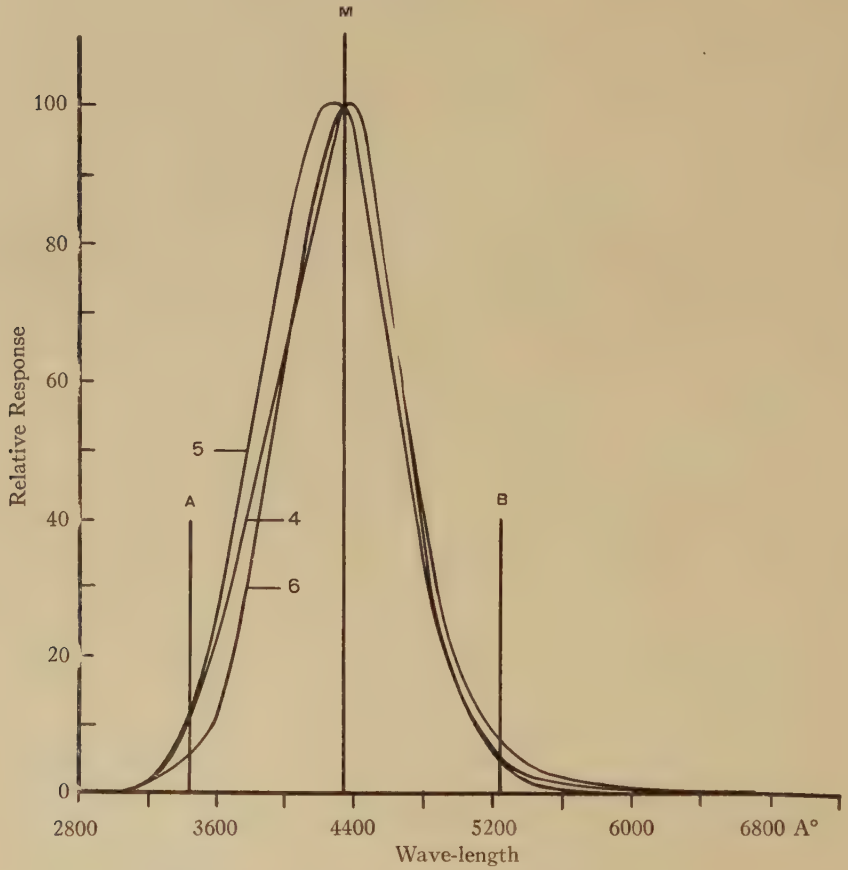

Fig. 2. Comparison of spectral sensitivities of photometers.
Curve 6: No. 7276—vacuum potassium (sensitized) cell—deck photometer.
Curve 5: No. 7275— " " " " —No. 1 sea photometer.
Curve 4: No. 7274—Gas-filled " " " —No. 2 " "

Each curve represents the relative response to an equal energy spectrum of the combined photo-electric cell and its diffusing disc of opal glass. For purposes of standardization the sensitivities are considered limited to the range between *A* and *B*, which are 900A° above and below *M*, the point of average maximum sensitivity.

$A = 3460\text{A}°$
$B = 5260\text{A}°$
$M = 4360\text{A}°$

present in a designated direction at a distance of one meter from the lamp (color temp. = 2835° K at 105.0 volts). The irradiation is expressed in microwatts/cm.[2] for each wave length interval of 100A° from 3200A° to 10,000A°. From these data the irradiation at the photometer window (placed one meter from the lamp) within the designated range of spectral sensitivity of the photometers was calculated and found to be 126 microwatts/cm.[2] By dividing this value by the photo-electric current produced, expressed in microamperes, the sensitivity of each photometer was found in terms of the irradiation necessary to produce a photo-electric current of one microampere. A summary of the standardization is given in Table I.

Changes in Emission of Photometers

Probably the greatest source of error during the measurements was the occurrence of changes in the emission of the photometers. It is unfortunate that there was no opportunity before the apparatus was assembled for testing the photo-electric cells in complete detail, as is recommended by Shelford (1930). When the "Atlantis" reached the United States and standardization was undertaken, the extent of the variability of the photometers was revealed. When the cells are irradiated in the darkroom by the standard lamp, the current emitted diminishes gradually during the course of the exposure.[4] This is particularly true of the gas-filled cell. This change of emission (formerly called "photo-electric fatigue") takes place chiefly within the first few minutes of exposure and rarely exceeds 10 per cent in magnitude (cf. Campbell and Ritchie, 1930; and Harrison, 1930).

Another more serious change in sensitivity was found to occur after an exposure of the photometer to intense irradiation (e.g. sunlight) for an hour or more. The response to the standard lamp following such treatment was greater—sometimes by as much as 30 per cent—than the response beforehand. Accordingly, when employed at sea, the deck photometer was probably less sensitive in the morning, after a night in darkness, than it was in the afternoon, after exposure to the noonday sun. Moreover, when either sea photometer had been used under water for an hour or more and then replaced on deck beside the deck photometer, it did not show the same ratio to the other instrument as before. It is hoped that in the future a satisfactory method can be devised for preventing the photo-electric current from surpassing the limit above which constancy is lost without reducing the range of intensities which can be measured.

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[4] The potential was always applied continuously to the cells for at least ten minutes before readings were begun, thus avoiding any "soakage effects" in the cable.

During the observations of 1931, no method for checking the photometers existed except by comparing them with each other. However, there is no evidence that the error due to changes of emission ever exceeded 30 per cent and probably was rarely greater than 15 per cent. The deck photometer was almost always used with its diaphragm in place (see Gall and Atkins, 1931) so that the photo-electric current was never large. Most of the submarine measurements were made with the vacuum cell photometer No. 1, which has been found to be more stable than the others. The values obtained for submarine irradiation in terms of percentages of the light falling upon the surface are considerably more accurate than the absolute measurements. Since the deck photometer and the sea photometer were compared before and after each series, corrections for any changes in sensitivity could be made.

For the work of 1932 a portable test lamp, similar to that employed by Poole and Atkins (1928), was constructed. It consists of a seasoned 50-candle-power auto headlight bulb mounted in a copper shade which fits snugly over the photometer window. The photo-electric emission produced in each photometer by this test lamp (run at 5.00 volts) is carefully observed just after standardization (cf. Table I). Assuming that any changes observed to occur in the emission produced by the test lamp represent corresponding changes in the sensitivity of the photometer to daylight, the absolute value of the irradiation measured at sea can be calculated. The constancy of the test lamp is vouchsafed by checking against the standard lamp from time to time.

Other Sources of Error

As light travels through the water, its nature becomes changed in two ways: First, its intensity is reduced; second, its spectral range is diminished by the progressive elimination of the longest and the shortest rays.[5] Any photometer with a broad spectral sensitivity would accordingly register a reduction in illumination due to a combination of these two effects, and the parts played by each would be indistinguishable. This difficulty can be removed to some extent by placing suitable light filters over the cells, or by using cells which are sensitive to only a narrow range of the spectrum. As will be seen from Fig. 2, the present method falls in the latter category. It must be borne in mind, however, that nothing can be said as to the distribution of the irradiation within the range of sensitivity. The error from this source is fortunately small due to the relatively narrow and steep shape of the sensitivity curve.

[5] For the relative parts played by pure absorption and by scattering, see Martin (1922) and Hulburt (1932).

A more serious chance of misinterpretation results from the fact that observations on the degree of penetration of an individual color do not give an accurate measure of the change in total irradiation in the sea. Our photometers are sensitive to the group of wave lengths which is believed to penetrate the farthest in clear sea water (Grein, 1914; Hulburt, 1932; Atkins and Poole, 1933; and Sawyer, 1933).[6] Accordingly, the change in total irradiation with depth is greater than is indicated by the measurements in the present paper. Ideally, each color should be investigated separately, and then the results added together to provide quantitatively accurate values for total irradiation. Although limited regions of the spectrum are chiefly responsible for influence upon certain vital functions (e.g. photosynthesis; cf. Klugh, 1930), the whole spectrum—or a large part of it—is undoubtedly effective in many other cases.

The measurements presented in this paper are subject to a number of other possible sources of error, common to all observations of this type, the importance of which it is difficult to estimate. A brief enumeration may be made as follows:

(1) Errors due to the shape and diffusing quality of the receiving surface of the photometer, and to the occurrence of "dark currents" in the measuring circuit. This last trouble has been reduced to small proportions in our apparatus, due to the excellent quality of the insulation (Gall and Atkins, 1931).

(2) Errors due to changes in the cloudiness of the sky, in the elevation of the sun, and in the condition of the sea surface. Possibly the sea may be sufficiently smooth at times so that the light rays are not scattered at the surface to a significant extent, but under other conditions diffusion is undoubtedly so great that the surface of the sea is, in effect, the source of irradiation. Since the nature and the extent of the action of the surface under different conditions is not thoroughly known, and since in certain cases the angle of incident light has been found unimportant (Atkins, 1932, p. 184), no correction for the altitude of the sun has been attempted in this paper (cf. Birge and Juday, 1930). Whatever effects the surface is found to exert, the results will differ according to the method of measurement used, and, accordingly, great

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[6] In determinations of the spectral absorption of sea water in which parallel rays chiefly are measured (cf. Sawyer, 1931) the rays of short wave length, which are scattered more than those of long wave length, are prevented by the apparatus from re-scattering back to the photometer as they do to some extent in measurements at sea. The transmission of the longer wave lengths would therefore appear relatively greater than under conditions where the scattered light was also measured. This fact should be borne in mind when making comparisons with other investigations. Suspended matter and stains may modify color selection profoundly (Shelford, 1929; Birge and Juday, 1931, 1932).

caution must be exercised in making comparisons with other investigations. In the present undertaking the values obtained for the upper water layers were checked in as many cases as possible with the neon lamp outfit (cf. Gall and Atkins, 1931).

(3) Errors due to the shadow of the ship and errors made in the estimation of the depth of the photometer. The ship was hove to with the stern directed as nearly as possible toward the sun, but in the work of 1931 it was not practicable to lower all the sails. The expanses of white canvas undoubtedly affected the deck cell, and in 1932 all the sails were lowered during measurements. The submarine photometer was suspended over the stern from the flagpole (in 1931) or from the end of the mizzen boom (in 1932). The heave of the ship caused a vertical movement of the photometer in the water, the amplitude of which was always noted (greatest extent, 3 m.), and the average depth recorded for each observation. This depth was corrected for horizontal displacement by multiplying by the cosine of the angle which the supporting cable made with the vertical for each observation. Since the cable probably was not straight under water but curved downward to some extent, this method of calculating the depth gives a slight over-correction (cf. Poole and Atkins, 1926).

(4) Errors due to the drifting of the ship into regions of water of different transparency in cases where horizontal distribution of suspended matter is irregular, and errors due to vertical changes in transparency caused by the diurnal migration of plankton. Observations on the actual effect of the plankton are discussed below.

These four possible sources of error are adequately discussed by Shelford and Kunz (1929), Shelford (1930), Poole and Atkins (1928, 1929), and Atkins and Poole (1933). There is no reason to suppose that the errors from these causes are any greater in this investigation than in those to which reference is made, and it is believed that the precautions taken, as outlined above, have actually reduced the magnitude of certain of the errors.

Observations

The observations made while crossing the Atlantic in 1931 are summarized in Table II. The complete curves for all of the series will be found in Figs. 3 and 4. The curves represent the irradiation at each depth expressed as a percentage of the light falling upon the surface and plotted on a logarithmic scale. The slope of each curve is therefore proportional to the value of its transmissive exponent. In each case the ratio of the photometers when the sea photometer was just over the surface is taken as 100 per cent. Measurements were ordi-

TABLE II

Summary of 1931 observations

Series No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Station No.	1002	1005	1007	1010	1013	1016	1019	1023	1029	1033	1038	1041	1053
Lat. N.	52° 17′	51° 17′	50° 09′	48° 27′	47° 04′	44° 59′	43° 13′	41° 45′	38° 13′	37° 27′	36° 56′	36° 55′	43° 27′
Long. W.	29° 46′	30° 03′	29° 57′	29° 58′	29° 59′	30° 08′	30° 10′	30° 33′	31° 39′	31° 44′	44° 52′	52° 41′	67° 39′
Date 1931	July 26	July 27	July 28	July 29	July 30	July 31	Aug. 1	Aug. 2	Aug. 4	Aug. 6	Aug. 12	Aug. 15	Aug. 24
Local sun time (24-hr. scale)	1133–1233	1235–1400	1205–1330	1130–1245	1115–1205	1110–1215	1100–1300	1103–1208	1253–1353	1223–1453	1545–1745	1055–1230	1010–1120
Av. altitude of sun	57°	46°	50°	60°	58°	60°	65°	61°	55°	54°	27°	64°	49°
Photometer No.*	1	1	1 N	1 N	1 N	1 N	1 N	1	2	1 N	1, 2	2	1 N
Av. transmissive exponent, *k*, over depth studied	0.10	0.10	0.061	0.058	0.051	0.052	0.049	0.055	0.044	0.053	0.046	0.031	0.12
Depth at which irrad. is reduced to 1%, *meters*	34	37	71	68	84	82	86	76	97	85	91	149	32
Range of irrad. on deck during series† (3460A°–5260A°), *microwatts/cm.²*	16,000–37,000	9,000–18,000	13,000–16,000	18,000–38,000	10,000–53,000	9,000–24,000	11,000–42,000	37,000–38,000	28,000–35,000	11,000–37,000	4,000–19,000	22,000–36,000	6,000–8,000
Sea (Douglas Sea Scale)	Moderate	Moderate	Moderate	Moderate	Moderate	Rough	Slight	Smooth	Moderate	Slight	Calm	Smooth	Rough
Sky	Clear	Overcast	Overcast	Few clouds	Many clouds	Many clouds	Few clouds	Clear	Few clouds	Few clouds	Few clouds	Clear	Overcast

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* N in photometer column indicates that the measurements just under the surface and in the upper water layers were checked with the neon lamp outfit.

† Some of these values may be too high due to the probable increase in sensitivity of the photometer after use in very intense light.

narily made every ten meters. The value for each depth is the average of at least three readings. All measurements below the surface have been corrected for "external reflection" and "internal reflection" by multiplying by the factor 1.09 (cf. Atkins and Poole, 1933, p. 135).[7] The first point on the graph gives the percentage beneath the surface, at a depth (1 to 1.5 meters) just sufficient to prevent the photometer from coming out of the water when the ship pitched. The value obtained represents the reduction of light due to loss at the actual surface and to the absorption by the layer of water above the photometer.

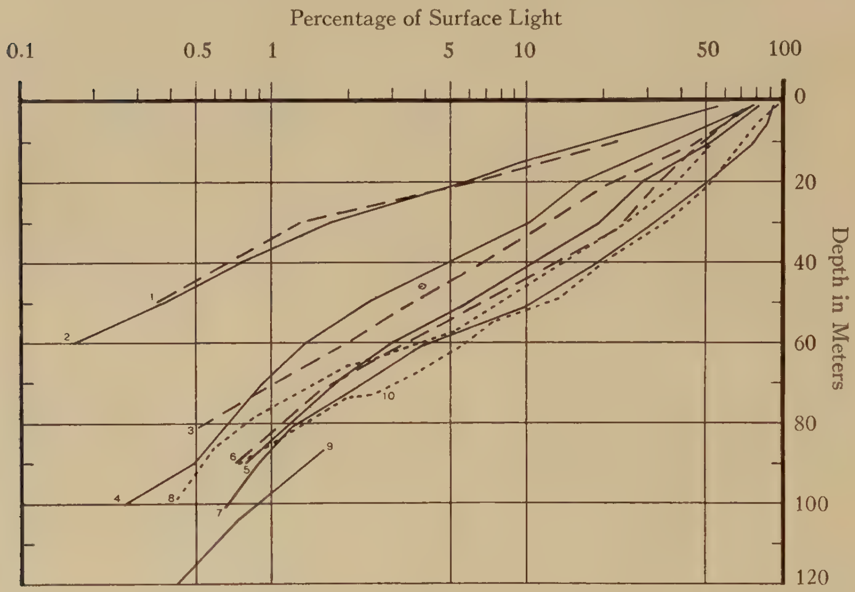

FIG. 3. Relation between depth and irradiation expressed as a percentage of the light just over the surface (logarithmic scale). The series shown (1 to 10) were made in a line running south on longitude 30° W. The figure at the end of each curve designates the series number of that curve. The broken lines are merely to aid in distinguishing individual curves from each other in cases where they cross.

The first ten series of observations were made on a line running north and south—approximately longitude 30° W. The observations were made in order from Series 1, the northernmost (lat. 52° N.), to Series 10, the southernmost (lat. 37° N.). It will be seen from Fig. 3 that the transparency of the water increases as one proceeds toward the south. The average transmissive exponent is the same for the first two series ($k=0.10$). For Series 3 the transmissive exponent is much reduced ($k=0.061$). The value diminishes more gradually thereafter, reaching a minimum in Series 9 ($k=0.044$). The values obtained in Series 5, 8, and 10 do not follow the sequence.

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[7] The use of this factor has resulted in values of 100 per cent or greater for certain of the measurements just beneath the surface. The source of error in these cases is not known.

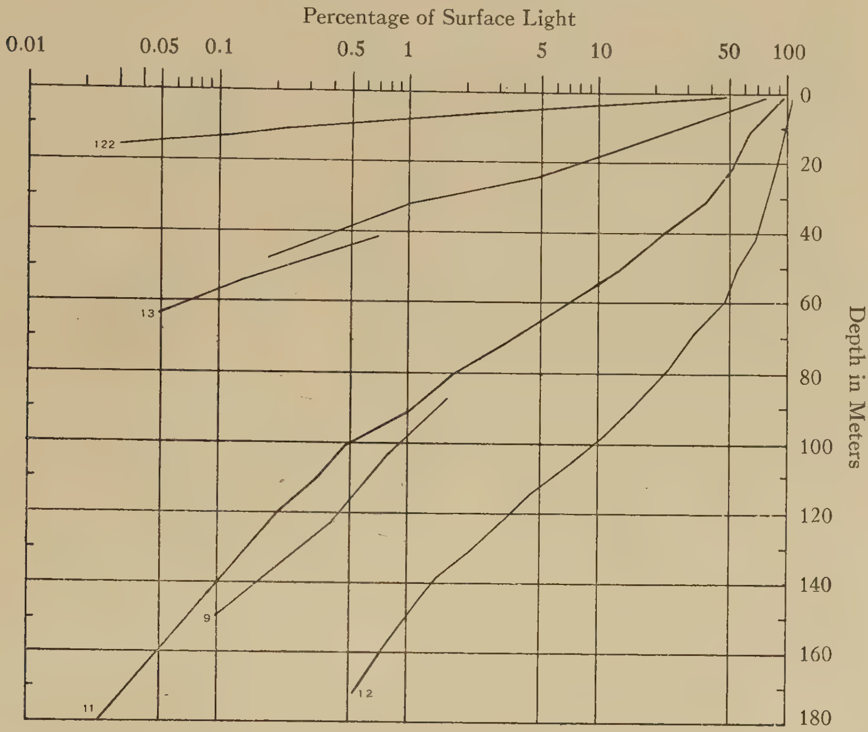

FIG. 4. Relation between depth and irradiation expressed as a percentage of the light just over the surface (logarithmic scale).

Series 9—near Azore Islands.
Series 11 and 12—Sargosso Sea.
Series 13—Gulf of Maine.
Series 122—Woods Hole Harbor.

Series 12 and Series 122 represent respectively the most transparent water and the least transparent water encountered. The anomaly of the curve for Series 12 starting above 100 per cent is discussed in a footnote in the text.

The sudden reduction in the value of the transmissive exponent found between Series 2 and 3 is coincident with the sharp division between northern water and southern water as marked by the northern branch of the Gulf Stream. Toward the south the surface water is warmer and in addition the stratum of warm water extends to a much greater depth (cf. Iselin, 1934). The lesser transparency of the northern water may be due to a greater amount of suspended matter (sediment and plankton) carried here from distant regions, or to a greater growth of plankton occurring in this region as a result of more favorable physical and chemical conditions.

A closer examination of the curves in Fig. 3 reveals that the differences in the transparency of the water at these ten stations are greatest within the upper 20 meters. In the first place great variation is found in the amount of light transmitted through the surface itself. The irradiation just under the surface (1 to 1.5 meters) varies from 57 per cent to 92 per cent. The lower values for the northern series may be due in part to the rougher weather experienced at those stations (cf. Atkins, 1932, p. 191). In the second place, the transmissive exponent of the subsurface water itself varies, for it is seen that the curves diverge rapidly from 1 meter to about 20 meters. From here on they are more nearly parallel. Since the sea was not smooth at any of these stations (except one), the change in altitude of the sun with latitude probably would not account for this change in transmissive exponent (cf. Atkins, 1932, p. 184). A greater amount of suspended matter in the upper 20 m. might account for the decreased transparency in this layer at the northern stations.

The curves for Series 11 and 12, made at stations 10° and 20° further to the west, are shown in Fig. 4. To facilitate comparisons with Fig. 3, the curve for Series 9 is repeated. The horizontal stray of the instrument was responsible for the fact that no greater vertical depth than 180 m. (calculated from wire angle) could be reached although the full 200 m. of cable was used. More irregularity was observed in the measurements at these great depths than normally characterized the shallower readings. This was undoubtedly due to fluctuations in the depth of the photometer which were not accurately reflected in the angle of the supporting cable. For the lower part of the series, the curves have been drawn as an average between the points rather than from point to point as in all other cases. The increased slope of the curve of Series 12 shows that the water at this station was more transparent ($k = 0.031$) than at any other station. However, the physical and chemical properties of the water were found to be the same as at the station where Series 11 was made (Iselin, 1934). The latitude of the two stations is practically identical. Since Series 11 was made in the late afternoon, whereas Series 12 was made at noon, the difference in elevation of the sun may have been responsible for the difference in transparency in this particular case. During both these observations the sea surface was smooth and probably did not diffuse the light nearly as much as in the cases discussed above.

The curves for the southern stations (Series 6 to 12) show a rather striking similarity in shape. The slope is steep at first, then becomes more gradual, and finally assumes a steep angle again. At the more easterly stations, the first change in slope occurs between 20 and 30 meters, the second change between 60 and 80. In Series 11 and 12 these changes fall respectively between 30 and 40 meters and between 100 and 140 meters. Evidently there exists an intermediate zone of decreased transparency which is found at greater depths at stations where the water is clearer. It is possible that this zone is similarly encountered at the northern stations, but that here it exists at a higher level and extends to the surface.

Series 13 was made in the Gulf of Maine under adverse weather conditions. The transparency is very slightly less than in Series 1 and 2. The remaining curve in Fig. 4 represents Series 122 made a year later in Woods Hole Harbor. The water here was found to be the least transparent encountered, light being reduced to 1 per cent at a depth of 8 meters. The curves of Fig. 4 therefore represent the extent of the range of transparencies observed during the two years work.

In 1932 eight series of observations were made in the Gulf of Maine. These are summarized in Table III. Curves plotted for the five series at Station 1287 are shown in Fig. 5. The loss at the surface, as indicated by the measurements at one meter, varies over a considerable range. Inspection of Table III shows that there is no simple relation between this loss and any one of the meteorological conditions. The value of this measurement probably results from a complex interaction of the elevation of the sun, the condition of the sky, and the state of the sea surface upon both reflection and refraction. However, the value of the transmissive exponent, from one meter down, varies very slightly during these four days. Diverse refraction at the surface may account for some of the differences observed, and the errors of measurement are probably responsible for others. The ship may have drifted into regions where varying amounts of suspended matter existed in the water. It is important to note that the diurnal vertical migration of zoöplankton has no measurable effect upon the transparency of the water in this case. The data presented elsewhere (Clarke, 1933) indicate the numbers of animals involved and the magnitude of their movements. The level of the zone of maximum abundance changes with the hour of the day. There are no corresponding changes in the transmissive exponent at the depths involved. Wherever steeper segments of the curve do occur, they do not correspond with zones of abundant zoöplankton.

Three series of measurements were made in Woods Hole Harbor from the power boat "Asterias." The data for these are presented in

TABLE III

Summary of Gulf of Maine and Woods Hole Series

Series	13	112	113	114	115	116	117	118	119	120	121	122
Station	1053	1285	1285	1286	1287	1287	1287	1287	1287	W. H. Harbor	W. H. Harbor	W. H. Harbor
Lat. N.	43° 27′	42° 34′	42° 34′	42° 30′	43° 04′	43° 04′	43° 04′	43° 04′	43° 04′	41° 31′	41° 31′	41° 31′
Long. W.	67° 39′	69° 37′	69° 37′	69° 37′	69° 24′	69° 24′	69° 24′	69° 24′	69° 24′	70° 40′	70° 40′	70° 40′
Date	Aug. 24 1931	July 9 1932	July 9	July 10	July 10	July 11	July 11	July 11	July 12	Aug. 16	Aug. 22	Aug. 22
Local sun time (24-hr. scale)	1010–1120	1302–1352	1907–1942	0652–0732	1526–1632	0805–0847	1203–1252	1837–1907	0612–0707	1149–1429	1029–1134	1339–1429
Av. altitude of sun	49°	64°	9°	16°	41°	29°	64°	15°	13°	61°	41°	50°
Photometer No.	1 N	1	1	1	1 N	1 N	1 N	1 N	1 N	1	1	1
Av. transmissive exponent, *k*, over depth studied	0.12	0.15	0.12	0.14	0.16	0.14	0.14	0.16	0.16	0.43	0.47	0.49
Irrad. at depth of 1 meter (% surface light)	78	100 *	69	78	87	55		72	55	44	48	48
Range of irrad. on deck during series (3460A°–5260A°), *microwatts/cm.²*	6,000–8,000	16,000–17,000	200–700	5,000–7,000	6,000–11,000	7,000–13,000	15,000–16,000	600–1,300	4,000–7,000	12,000–15,000	11,600–12,400	11,800–12,400
Sea (Douglas Sea Scale)	Rough	Slight	Moderate	Moderate	Slight	Rough	Moderate	Moderate	Slight	Calm	Calm (Glassy)	Calm
Sky	Overcast	Few clouds	Few clouds	Few clouds	Overcast	Overcast	Clear	Few clouds	Clear	Clear	Clear	Clear

* The explanation for this value, which is obviously too high, is not known (see footnote in text).

Table III and the curve for Series 122 is shown in Fig. 4. In this series measurements were made every meter to a depth of 16 meters. Since the water in the harbor is so rapidly changed by swift tidal currents, considerable fluctuations in the amount of sediment present undoubtedly occur. In Series 121 it was possible to measure the irradiation with the photometer suspended so that its window was only a few centimeters below the surface. The irradiation was found to be 84 per cent of that recorded a few centimeters above the surface. Two hours later when Series 122 was made, a gentle breeze had sprung up and it was not possible to make another measurement as near the surface as before. The transmissive exponent for the whole series was slightly increased, but the irradiation at one meter remained the same.

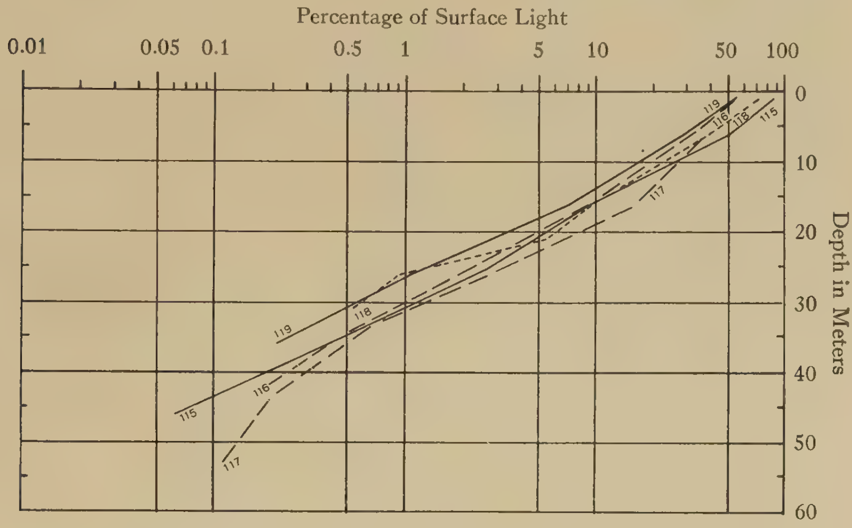

Fig. 5. Relation between depth and irradiation expressed in percentage of light just over the surface (logarithmic scale). The series shown (115 to 119) were all made at Station 1287 in the Gulf of Maine. The broken lines are merely to aid in distinguishing individual curves from each other in cases where they cross.

DISCUSSION

A thorough review of the literature on the transmission of solar radiation through water has recently been made by Atkins (1932). Accordingly, no systematic comparison of the work of previous investigators will be attempted in the present paper. However, the general relationship between observations reported here and comparable investigations will be discussed briefly.

Comparisons can be made directly with the work of Poole and Atkins since our photometers have been standardized against theirs (see Table I). The values for surface loss vary within the same range in both investigations. The transmissive exponents found to characterize the water at Stations 1002 and 1005 and at Stations 1053 and 1287 are of the same magnitude as those observed by Poole and Atkins (1929) in the English Channel.

The measurements at stations farther to the south give transmissive exponents lower than any previously reported using the photoelectric cell. Employing photographic methods, Grein (1914) detected blue-violet light in the Mediterranean at 1500 meters; and Helland-Hansen (1931) found blue light at 500 meters in the Sargasso Sea.[8] Shelford and Gail (1922) reported blue light at 120 meters in Puget Sound amounting to about 0.2 per cent of the surface light. This indicates a transparency approximately equivalent to our Series 11. The transmissive exponent recorded for Series 12 ($k = 0.031$) approaches the value regarded by Atkins (1932, p. 185) as possible for the clearest ocean water. The values for the upper 59 meters in this series are:

meters	k
1–21	0.010
21–31	0.013
31–41	0.011
41–49	0.028
49–59	0.015

The transmissive exponent for the first 41 meters is lower than the minimum for pure water ($k = 0.015$) according to Sawyer (1931). This apparent anomaly may be due in part to the fact that Sawyer's measurement is diminished by the loss of light scattered in its passage through the water. In our measurements this loss is compensated to some extent by light which is scattered back into the cone of radiation received by the photometer. It does not seem likely that this effect could account for the whole discrepancy. Evidently, the measurements include some error. The fact that the value just under the surface is greater than 100 per cent also indicates this (see footnote above). However, one can hardly escape the conclusion that the transparency of the water at this station was exceptionally great—perhaps near the maximum possible.

The least transparent water observed—in Woods Hole Harbor—was found to have a transmissive exponent comparable to that reported by Poole and Atkins for certain measurements in the English Channel, especially those made near shore in the Plymouth region. The water of many of the Wisconsin lakes investigated by Birge and Juday shows a similar degree of turbidity. The transparency reported by Shelford (1929) for San Juan Channel is intermediate in value between that found in Woods Hole Harbor and that found in the Gulf of Maine. The present measurements, therefore, fall in line with those made by other investigators and, to a certain degree, extend our knowledge of the penetration of light into the sea.

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[8] See Atkins and Poole (1933, p. 154) for discussion of the conditions in the Sargasso Sea. Dr. Atkins informs me also that the color of the water on the Forel scale is 2 in this region as contrasted with 6 in the region where our first series was made.

SUMMARY

1. Measurements of the penetration of daylight into the sea were made in the mid-Atlantic, in the Gulf of Maine, and in Woods Hole Harbor, using the photo-electric method described previously. A four-conductor insulated cable and an electrically operated internal shutter have been added to the No. 1 sea photometer.

2. All three photometers exhibit essentially the same spectral sensitivity. For purposes of standardization the range of sensitivity is considered to extend from 3460A° to 5260A° with a maximum at 4360A°.

3. The photometers have been standardized against a 1000-watt Mazda lamp, the value of the irradiation from which was limited to that occurring within the spectral range of the photometers. The results of standardization are presented in Table I.

4. The changes in emission of the photometers and other possible sources of error are discussed, particularly those not dealt with by previous investigators.

5. The observations made approximately on longitude 30° W. reveal an increasing transparency of the water, particularly of the upper 20 meters, toward the south. Toward the north, the transparency is slightly greater than that found in the Gulf of Maine (transmissive exponent, $k = 0.15$).

6. The most transparent water encountered was in the Sargasso Sea where measurements were made down to 180 meters. In Series 12 the average exponent was $k = 0.031$, and values of $k = 0.015$ to $k = 0.010$ were obtained in the upper water layers. The least transparent water was found in Woods Hole Harbor ($k = 0.49$).

7. Examination was made of the extent to which the altitude of the sun, the condition of the sea surface, and the vertical distribution of the zoöplankton affected the degree of penetration of light. Five series of observations under varying conditions at one station in the Gulf of Maine revealed no significant change in transparency.

8. The measurements of submarine irradiation are found to be commensurate with those of previous investigators, but certain significant differences are pointed out.

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Contribution No. 27

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Fourteenth Annual Meeting, 1933)

THE DEVELOPMENT OF OUR CONCEPTION OF THE GULF STREAM SYSTEM

C. O'D. Iselin

To any student of the physical oceanography of the North Atlantic Ocean, the current-system, popularly known as the Gulf Stream, is a problem in more ways than one. Not least among the difficulties is the confusion in the literature over the definition of the four chief names under which this current-system or parts of it are now known, namely, Florida Current, Gulf Stream, North Atlantic Drift, and Atlantic Current.

It might seem at first glance that the question of terminology was an unimportant one, but anyone reading back through the many papers that have been written about this mighty current will soon see that part of the misunderstanding is due to the fact that the term Gulf Stream means one thing to one writer and another to another. The various names by which the parts of this current are known have been used loosely largely for the reason that so little was known about the structure of its different sectors and about the forces acting to move the water along its great length. But we have now come to the time, mainly because of the sections made during the past two years by the *Atlantis*, when we have found out something about the structural changes the current undergoes between the Straits of Florida and mid-ocean. And for the eastern half of the ocean, we can turn to Helland-Hansen and Nansen for an authoritative summary of what is known about the complicated branching of the Gulf Stream, wisely called by them the Atlantic Current. Even though we are still far from understanding the forces which act to keep the water moving, it is clear from the *Atlantis* sections that on the basis of structure the stream naturally divides itself into three parts, and for this reason it would seem that the time has come to adopt a uniform and clearly defined terminology. Fortunately, there is no need to introduce new terms. It is mainly a question of defining more exactly those now in use among oceanographers and to hope that the same usage will be gradually adopted in all languages.

There is no question but that the term Gulf Stream among English-speaking people (and largely also in other languages) is popularly used for the whole current, under the impression that there is a warm river of water flowing out of the Gulf of Mexico, up along the eastern coast of the United States towards the "tail" of the Grand Banks, and thence to Europe and the Arctic. Historically also the name holds the lead in the literature of the last century. Thus in science the term should be continued and, as we shall see, the only safeguard necessary to avoid confusion is to speak of the "Gulf Stream system" as meaning the whole of the current, including the branches.

The controversy over the Gulf Stream has waxed and waned ever since the printing of Lieutenant Maury's wind and thermal charts in 1852. In 1870 a sort of crisis was reached with the publication of Petermann's paper, and his vigorous denial of the more moderate views of the leading English and American scientific men brings out clearly the two main conceptions of the Gulf Stream's influence in the eastern North Atlantic. Since by this time surface-salinity determinations had been made and a number of deep temperature-stations occupied, 1870 can be taken as the beginning of the modern scientific investigation of the Gulf Stream.

Until this time, the current had been purely a map-maker's problem. How far be-

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Contribution No. 27 from the Woods Hole Oceanographic Institution yond the Grand Banks should the arrows be continued which show the strong flow out of the Gulf of Mexico? Rennell in 1832 had drawn them as far as the Azores and this was considered rather an extreme view. But with the increasing interest in meteorology and arctic exploration, scientific men soon came to see that the climate of northern Europe, and indeed also of such places as Iceland and Spitzbergen, was much milder than the average for similar latitudes. Sea-surface temperatures also showed relatively warm water extending much further north in the eastern Atlantic than elsewhere.

Then came Petermann and his able explanation of the whole situation. He claimed that a branch of the Gulf Stream flowed northward through the Norwegian Sea and even into the Arctic Ocean. With German thoroughness he supported his views by collecting all available surface-temperatures and constructing two thermal charts, one for July and the other for January. In his zeal to put forward a completely new theory he perhaps went too far, for he showed the Gulf Stream continuing as far as 82° north latitude and consisting in the arctic of all the water warmer than 2° at the surface.

Those opposed to these views (Findlay, Carpenter, and others) argued that the Florida Current did not have sufficient volume to extend to Europe and brought forward the prevailing southwest wind to explain the relatively warm water off Ireland and in the Norwegian Sea. Petermann replied that the wind was not always from the southwest and pointed out the relative warmth of the sea in winter, the great distance the wind had to move the water, and, more important, that the warmer water often remained in well-defined bands.

If Petermann had not called his northern relatively warm currents the Gulf Stream, his ideas would have had even more influence than they did, for he presented a very strong case. As it was, his opponents could always point to the immense distance between Florida and Norway and to the narrowness and shallowness of the Straits of Florida. It seemed preposterous, as indeed we now know, to think that all the warmest surface-water in the northeastern Atlantic had passed through the Straits of Florida.

Here we see the main pitfall that has beset many meteorologists and students of oceanic circulation, who have used the term Gulf Stream when writing of the warm waters found off the coast of Europe. The tendency has been to convey the impression that the Gulf Stream is the chief source of warm water and the main means by which heat is transported northward.

In 1901 Dickson published another notable series of charts showing the monthly surface-temperatures in the North Atlantic. By this time the increasing information about the ocean had all tended to reduce the relative importance of a river-like Gulf Stream and to bring forward the wind as an agent for transporting warm water towards the shores of Europe. Dickson's paper perhaps represents the furthest possible advance in studying the course of a great current-system by means of surface-temperatures alone. Similar work since his time has produced little that is conclusive so far as pointing to the path of the various branches of the Gulf Stream system for the reason that the currents are deep phenomena and often hidden by the wind-controlled surface-movements.

As an example of recent work based on surface (in this case 0-100 meters) temperature and salinity conditions, I might mention the papers of LeDanois which have appeared frequently since 1923. Starting with the periodic appearance of relatively warm saline water in the Bay of Biscay, he has developed his "transgression" theory which, as we shall see, is a good exposition of what actually happens, but he has received little attention partly because he has stuck to the term Gulf Stream to describe the variable extensions of tropical water in the eastern Atlantic.

The immense influence on scientific and popular opinion of the many atlases of the North Atlantic which have appeared in the last 50 years should not be overlooked. They all give the impression that the Gulf Stream along the American coast is at least twice too wide and that it increases in width until by mid-ocean the current-lines often have spread out fanwise to a width of 600 miles or more. The whole of this familiar system of stream-lines or arrows has usually gone under the name of Gulf Stream.

This brings us to our first fundamental distinction. The word current in oceanography should, in my opinion, be reserved for the more powerful and permanent move-

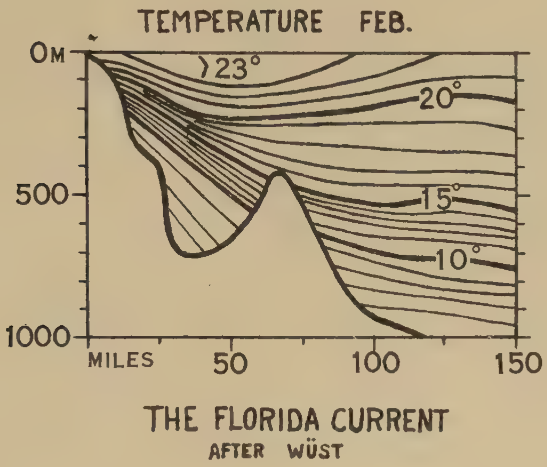


Fig. 1

ments which extend deeper than the drift set up by variable winds. Moreover, the word should include the whole depth of the moving water and not just the surface aspect of it. Thus a steady wind, for example, the trades, can set up a current, but the cyclonic type of winds found east of the Grand Banks, although prevailingly from the western quadrant, set up only a drift in that the movement must be shallow and varying in direction.

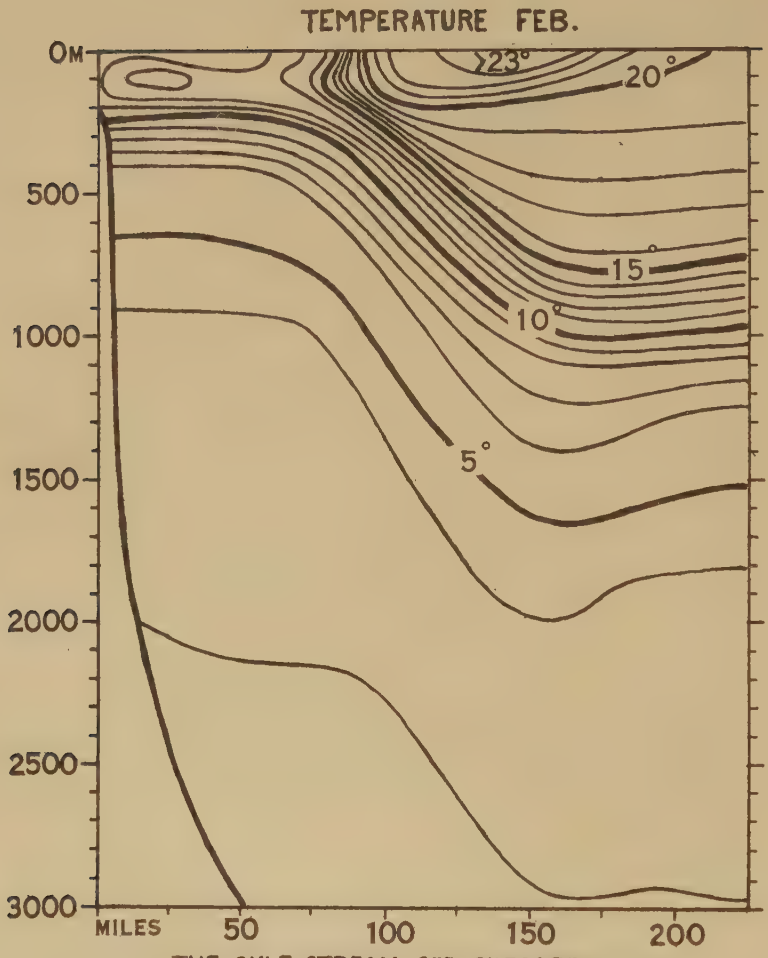
With this in mind we can see that atlases and so-called "current-charts" usually show the sum of the permanent currents and the prevailing drift. In the eastern North Atlantic, especially, there are both types of movement tending to bring relatively warm water northward, but the name Gulf Stream should only be applied to the deeper continuous lines of current. In fact, even this use is misleading and most authors now prefer to use the terms North Atlantic Drift and (North) Atlantic Current for the two types of movement found in this region.

We can see now what it was that Petermann from surface-observations thought to be a continuation of the Gulf Stream. The North Atlantic Drift, being as widespread as the wind, causes a general slow movement at the surface, but wherever a north-flowing branch of the Atlantic Current carries some of this surface-water northward, especially if the wind is not strong, the warmest water will be found at the surface in definite bands.

The branch of the Atlantic Current which enters the Norwegian Sea is known as the Norwegian Atlantic Current. Other branches have also been named, but their course is not so well known, being more or less masked by the North Atlantic Drift, so that only through subsurface observations can the intricate system of currents in the eastern North Atlantic be disclosed.

Since the name Atlantic Current is well established (Nansen, Helland-Hansen, and others) among oceanographers and nicely avoids the connotation of heat forever associated in the term Gulf Stream, its use should be continued and among our problems there remains the question of where the Gulf Stream stops and the Atlantic Current begins, for the latter is the continuation of the former. But before going into this point let us turn our attention briefly to the Gulf Stream proper.

Between Cape Hatteras and Nova Scotia we now have eight Atlantis sections and two others from other modern expeditions. These all show a sharp transition-zone between the relatively warm and motionless water-layers of the Sargasso Sea and the colder band of Slope-water which bathes the American continental slope. The latter averages about a hundred miles wide, while the transition zone is usually not more than 65 miles wide. The current is, of course, situated between the Slope-water and the Sargasso water and across its width the isotherms drop on an average of 600 meters. As revealed

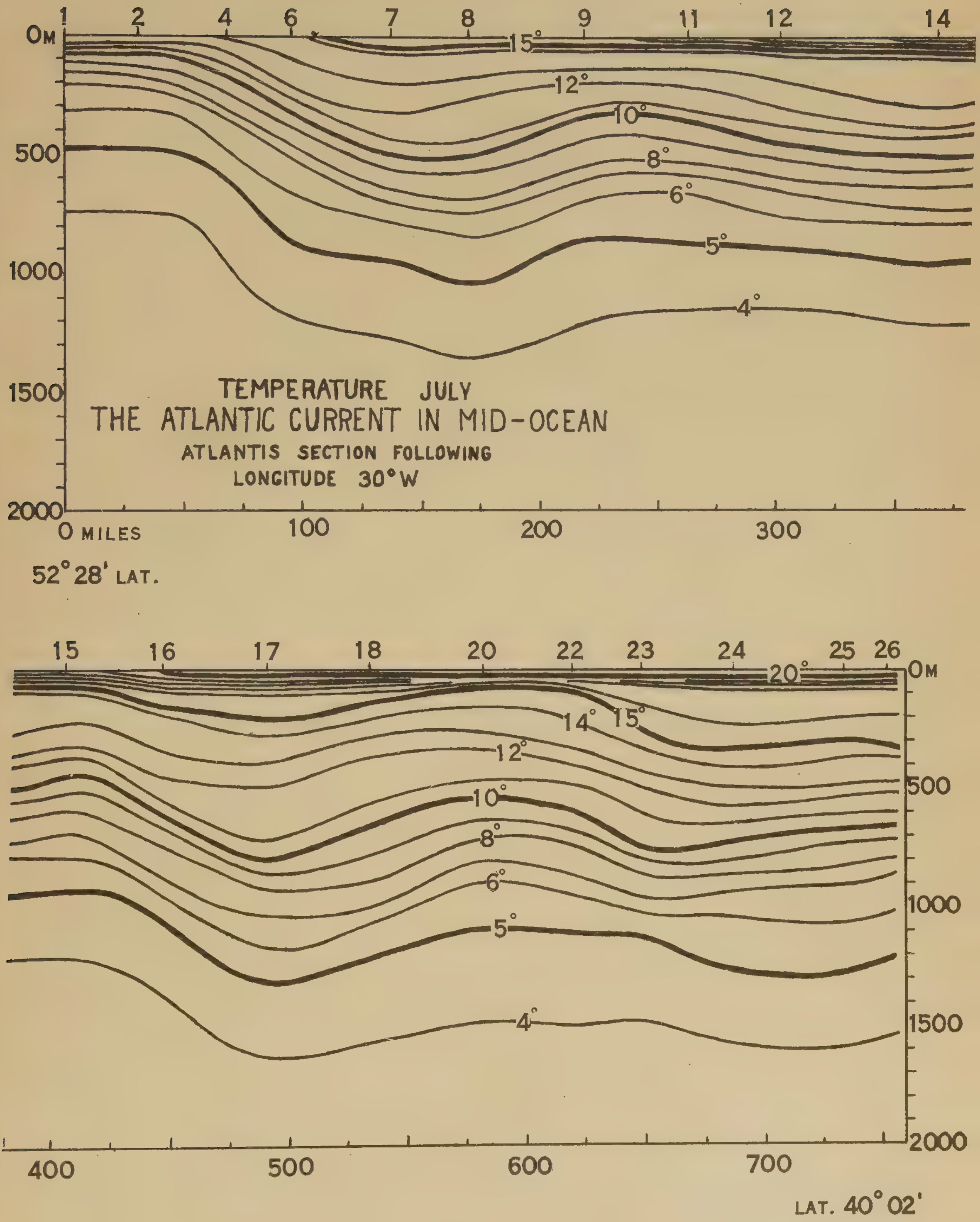


THE GULF STREAM OFF CHESAPEAKE BAY
FROM ATLANTIS OBSERVATIONS

Fig. 2

by the sloping isotherms, the current along this sector of the Gulf Stream extends down to at least 2,000 meters and only in the surface layer (0-100 m) can any water be found which is warmer than that at the same depth in the Sargasso Sea. In short, the narrow boundary between the Slope-water and the Sargasso water is seen in the available sections (through a study of the temperature-salinity ratio) to consist only of a mixture of these two kinds of water, and of all the moving water perhaps only a small per cent has come out of the Gulf of Mexico.

On the other hand, sections across the Straits of Florida (Wüst 1924) when studied closely reveal a different state of affairs. In the first place, because of the shallowness of the passage (700 meters) the depth of the Current is very much less. Even along the coast between Florida and Cape Hatteras the Current must remain relatively shallow for the reason that it flows over the Pourtales Plateau which is never much more than 1,000 meters deep. Secondly, the temperature-salinity ratio at mid-depths (500 meters) is quite different in the Straits of Florida than north of Cape Hatteras. Although the study now being made of this problem is not concluded and the Atlantis is only now making the necessary observations, I do not hesitate to say that little water which flows out of the Gulf of Mexico remains in the Current by the time Cape Hatteras


TEMPERATURE JULY
THE ATLANTIC CURRENT IN MID-OCEAN
ATLANTIS SECTION FOLLOWING
LONGITUDE 30°W
1
2
4
6
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11
12
14
0M
500
1000
1500
2000
15°
12°
10°
8°
6°
5°
4°
0 MILES
100
200
300
52° 28' LAT.
15
16
17
18
20
22
23
24
25
26
20°
14°
15°
12°
10°
8°
6°
5°
4°
0M
500
1000
1500
2000
400
500
600
700
LAT. 40° 02'

Fig. 3

is reached. The Current is of course continuous, but gradually the Gulf of Mexico water becomes replaced at all depths by Sargasso Sea water or a mixture of this and Slope-water. The secret of the matter probably is that the flow through the Straits of Florida has nothing to do with the more fundamental convectional current north of Cape Hatteras, the real Gulf Stream.

For these and other reasons it seems desirable to utilize the term Florida Current, not only for the flow through the straits, but also northward to the point where the Current runs off the Pourtales Plateau and into deep water. Between this point and the tail of the Grand Banks we can clearly use the name Gulf Stream, because the deep compact character remains unchanged along this sector. We may note here that Krümmel and other German writers have urged the adoption of the name Florida Current for the whole of the stream on the grounds that it is older than the term Gulf Stream, but the latter has become so universally used that it would be almost impossible to discard it now.

How far beyond the tail of the Grand Banks the Current continues before it branches is as yet not surely known. The Atlantis section of 1929 along longitude 30° W shows that by mid-ocean there are at least three well-defined branches, each narrow and deep. Thus somewhere beyond the Grand Banks the transition between the Sargasso Sea conditions and that of the northern water ceases to take place in one sharp lift of the isotherms and becomes a series of step-like rises, each band of sloping isotherms (current) being separated by broad strips with relatively horizontal conditions (still water). Beyond the point where the Gulf Stream branches we should speak of it as the Atlantic Current, for the flow is of a different character.

There is one more conception which I wish to bring forward and it will take us again to the work of LeDanois and his transgression-theory. I have suggested that the Gulf Stream system beyond Cape Hatteras (that is, the Gulf Stream and the Atlantic Current) marks the boundary between the Sargasso Sea and the slope or northern waters. Moreover, I have suggested that the Gulf Stream system is not like a river consisting always of the same particles of water flowing in a set channel. If at any time there is more Sargasso (tropical) water, the position of the abrupt northern boundary will change, that is, the path of the Gulf Stream, or more likely the Atlantic Current, will be shifted northward. These slight lateral movements of the currents and especially of the eddy-like terminal branches of the Atlantic Current can often cause warm water to flood in over the banks and shallow seas formerly occupied by northern water and produce the conditions called "transgressions" by LeDanois. Ordinarily, the Gulf Stream marks the limit to which tropical water can spread.

In conclusion, we have attempted to show that logically it is justifiable to split up the Gulf Stream system into three parts on the basis of the structure of the current when examined in cross-section, and that historically there is good precedent to call these the Florida Current, the Gulf Stream, and the Atlantic Current. The latter is branched and the course of all of its branches have not yet been worked out, as the flow is masked by the shallow North Atlantic Drift.

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ARSENIC IN SEA WATER

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FROM THE WOODS HOLE OCEANOGRAPHIC INSTITUTION[1]

Interest in the occurrence and quantity of arsenic in sea water centers chiefly around its relation to phosphorus: first, the possibility of its utilization by diatoms or other organisms, in place of or in addition to phosphorus; and second, the possible interference of arsenate in the regular routine determination of phosphate so frequently carried out upon sea water samples.

Arsenic has long been known to occur in the sea. Quantitative determinations by Gautier (1), and others (2), using laborious methods, indicated its presence in amounts of the order of 10–100 milligrams per cubic meter.

Gautier found that in surface water (from near the coast of Brittany) containing 10 mg./m.^{3} of total arsenic, 90 per cent was present in inorganic form. At one deep-sea station he found 25 mg./m.^{3} of As at a depth of 10 meters, 10 mg. at 1335 m., and 80 mg. at 5943 m. He was inclined to attribute the high value at the bottom to volcanic influences and the moderately high value in the upper level to some factor associated with diatom growth. Others, following these limited results, have regularly assumed an increase of arsenic with depth.

Chapman (3), while studying the arsenic content of shell-fish and other marine organisms, made sixteen determinations on sea water samples from near the Nore Lightship. He reports 0.14 to 1.0 parts per million, with an average of 0.33; *i.e.*, more than 300 mg./m.^{3}!

Atkins and Wilson (4), in a study of the methods for the determination of phosphate, silicate, and arsenic, have shown good reason for assuming that while arsenate interferes in the colorimetric estimation of phosphate by producing a similar color, arsenite is without effect; furthermore, that since total As is frequently in larger amounts than the colorimetric method indicates phosphate, most of the arsenic in sea water must be present in some other form, presumably arsenite.

It has been reported, on the basis of culture experiments, that arsenate can substitute for phosphate, to a certain extent, as a requirement for the growth of diatoms, but it is unknown to what extent this actually takes place in the sea.

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[1] Contribution No. 29.

Data on the total arsenic content of sea water have been very scarce, and since no application seems to have been made of the Gutzeit method, the superiority of which over the older Marsh method has been frequently demonstrated, the present authors undertook to apply the more recent procedure to a number of sea water samples.

These were obtained during the summer of 1932 in the course of the regular collecting cruises of the vessels, "Atlantis" and "Asterias," of the Woods Hole Oceanographic Institution, from a number of inshore stations in the Gulf of Maine, to the south of Martha's Vineyard, off the entrance to New York Harbor, etc. In addition, two deep-sea stations are listed in the tables, No. 1349, at 38° 58′ N., 64° 30′ W., and No. 1370, at 35° 55′ N., 72° 46′ W.

TABLE I

Data on Concentration of Arsenic in Sea Water Taken at Different Depths at Various Stations

Gulf of Maine

Station	Depth	As	Station	Depth	As	Station	Depth	As
	m.	*mg./m.³*		*m.*	*mg./m.³*		*m.*	*mg./m.³*
P-1	0	7	H-8	0	17	1333	0	15
	60	15		10	22		9	15
F-15	0	10		20	12		17	22
	10	17		30	13		26	13
	50	21		50	10		35	16
	75	15		75	15		44	16
	100	10		100	17		52	13
G-12	0	9		160	18		65	16
	10	17	1329	0	9		87	12
	30	11		10	11		186	16
	50	10		20	12	1335	0	9
	75	15		30	10		10	11
	105	6		40	29		20	10
G-11	0	9		50	17		30	10
	10	10		60	14		40	7
	20	14		75	11		50	10
	30	12		100	17		60	10
	50	24		240	20	1337	0	15
	75	13	1331	0	17		10	12
	110	10		10	18		20	10
H-3	0	17		19	20		30	10
	10	16		29	15		40	13
	20	16		39	15		50	10
	30	14		48	11		60	11
	50	16		58	16			
	75	(28)		72	18			
	100	10		97	18			
	115	13		194	12			

TABLE I—*Continued*

N.E. of Cape Cod

Station	Depth	As
	m.	*mg./m.*^{3}
B′-5	10	20
	20	20
	30	25
B′-20	0	18
	20	22
	100	20
	243	25

Off Entrance of N. Y. Harbor

Station	Depth	As
	m.	*mg./m.*^{3}
1297	0	9
	60	22
1298	0	16
	70	26

South of Martha's Vineyard

Station	Depth	As
	m.	*mg./m.*^{3}
A-4	0	10
	10	17
	15	11
A-8	0	14
	10	14
	20	17
	26	8

South of Martha's Vineyard (cont.)

Station	Depth	As
	m.	*mg./m.*^{3}
A-25	0	10
	10	11
	20	21
	30	7
	40	10
	50	10
A-28	0	11
	10	21
	20	14
	30	21
	40	(9)
	50	(27)
	57	10

Long. 38° 58′ N.
Lat. 64° 30′ W.

Station	Depth	As
	m.	*mg./m.*^{3}
1349	0	28
	200	17
	300	12
	400	20
	600	12
	800	16
	1000	17

Long. 38° 58′ N.
Lat. 64° 30′ W.
(cont.)

Station	Depth	As
	m.	*mg./m.*^{3}
1349	1200	18
	1600	20
	2000	15
	2500	16
	3000	20
	4000	15

Long. 35° 55′ N.
Lat. 72° 46′ W.

Station	Depth	As
	m.	*mg./m.*^{3}
1370	0	54
	50	22
	100	21
	200	20
	400	20
	600	22
	800	(31)
	1200	16
	1600	17
	2000	16
	2500	15
	3000	13

The Gutzeit method (5), as now commonly employed in the analysis of foods etc., was used without serious modification, concentrating 100 cc. of the water to small volume before use. The length of the stain on the $HgBr_2$-paper was compared for each sample with those resulting from standard solutions of arsenic. The results, with descriptions of the stations and the depths in meters from which the samples were taken, are given in the tables. Figures indicate milligrams of arsenic per cubic meter of water, *i.e.*, parts per billion.

The results from Station K-2, in Buzzards Bay, represent a series of samples taken successively from the same station over a period of several hours and afford, perhaps, a fair indication of the accuracy of the method. Only one variation of more than 6 mg./m.^{3} is found at corresponding depths, and this range must include not only experimental errors but also any actual variation in the water during the time.

In the case of the shallow stations there seems to be a distinct tendency for an increase in arsenic a short distance below the surface, usually with a decrease again at greater depths. This tendency is far from consistent, however, which is to be expected if it is associated with the variable conditions of the phytoplankton, or diatoms, from one station to another.

The two deep stations present a different problem, with their unusually high surface values, which may, perhaps, be purely accidental. But, more important, there is no regular or significant increase of arsenic with depth. Contrasting this with the case of phosphorus, which, at such a station, would show a ten-fold increase, at least, between the surface and bottom, shows that the two elements are utilized in very different manner.

TABLE II

Data on Concentration of Arsenic at Station K-2 in Buzzards Bay at Different Times of Day

Depth	Time				
	6:25 A.M.	9:31 A.M.	12:37 P.M.	3:43 P.M.	6:49 P.M.
m.	*mg./m.*^{3}	*mg./m.*^{3}	*mg./m.*^{3}	*mg./m.*^{3}	*mg./m.*^{3}
0	15	16	11	13	13
5	15	14	14	16	14
10	10	16	12	15	20
15	13	17	15	11	

Out of 155 determinations made, 140, or 90 per cent, fall between 9 and 22 mg./m.^{3} of As. This small range may, we think, be taken as the normal. Only seven samples were found with more than 25 mg. The average and median of all determinations, irrespective of depth or locality, were each 15 mg./m.^{3}.

The data yield two important general results:

1. Failure to bear out the previous findings of Gautier, who reported a considerable increase in As concentration with depth. On the contrary, although a small amount of the As may be removed from the water at certain stations by diatoms etc., this process is not nearly so important or general as in the case of phosphorus.

2. Corroboration of the assumption of Atkins and Wilson that the greater portion of the As in sea water is present in the form of arsenite.

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THE BUFFER CAPACITY OF SEA WATER[1]

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The titration of sea water in order to measure its excess of base, or what may be called its buffer capacity for acids, is not only of interest from the standpoint of oceanography but also of practical value in the detection of acid wastes present in small quantities (Thompson and Bonnar, 1931). Several methods have been employed. Wattenberg (1930) has described a method in which an excess of acid is added to the sea water, CO_2-free air is drawn through the boiling mixture until all CO_2 is removed, and then the water is back-titrated with standard barium hydroxide solution, using a mixture of brom cresol green and methyl red as indicator. Greenberg, Moberg, and Allen (1932) use a method of direct titration with standard acid, using methyl orange as indicator. Both of these methods, and others of which they are typical, require the use of rather elaborate precautions and of more or less cumbersome apparatus.

Of recent methods, that of Thompson and Bonnar (1931) possesses certain distinct advantages. It is rapid and employs apparatus easily handled on board ship. It is especially good in that results are practically independent of the CO_2 tension of the sample.

The principle of the method is simple. A sufficient volume of standard HCl is added to a measured portion of the sample to produce an acidity near the middle of the useful range of brom phenol blue. From this the milliequivalents of hydrogen ion per liter remaining unneutralized are calculated. This value subtracted from the quantity of H ion added per liter gives what might be called the apparent buffer capacity of the water. This value, however, must be corrected for the effect of salts upon hydrogen ion activity and upon the dissociation of the indicator. The correction is made by carrying out the same procedure on a sample of carbonate-free, approximately neutralized sea water of the same salinity as the unknown sample. Subtracting the acid required by the carbonate-free water from that required by the sample gives the buffer capacity of the latter.

It seemed to us that if standard values for the amount of acid required to bring carbonate-free sea water of any given salinity to any given pH could be established, the method would be made much more useful, especially since the difficult part of Thompson and Bonnar's method is the preparation and preservation of neutral, carbonate-free water. Our experience has led to the conclusion that such standard values can be obtained. Sea waters from distinctly different regions, whether clear or cloudy (provided they are filtered before use) will yield the same results within the limits of observational error, when rendered truly carbonate-free.

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[1] Contribution No. 31 from the Woods Hole Oceanographic Institution.

The method of preparing the "neutralized" water has been slightly modified. Concordant and reproducible results can be obtained only when certain precautions are rigidly adhered to. Filtered sea water, sufficient to half fill a large Pyrex boiling flask, is treated with concentrated H_2SO_4, drop by drop, until a test portion gives a color with brom phenol blue approximately equivalent to that of a Clark and Lubs standard tube of pH 3.4. The water is then boiled for about six hours under a Pyrex reflux condenser, while a stream of CO_2-free air is rapidly drawn through it. The reflux condenser is then removed, and while the water is still boiling, carbonate-free NaOH of about 0.07N strength is added in small portions until a sample of the water quickly cooled to room temperature shows a pH of 6.6 with brom thymol blue. The approach to the condition of near neutrality is followed from time to time by titrating a cooled 25-ml. portion of the water with 0.01N NaOH, using brom thymol blue as indicator. As noted by Thompson and Bonnar, the occurrence of an alkaline condition in the water at any time during the approach to neutrality vitiates the preparation. CO_2 will be taken up so rapidly that the water, even though it is acidified as soon as the slight alkaline condition is discovered, will show acid requirements that are too high.

Measurements are made, after the preparation has been cooled to 20° C., upon various known dilutions with distilled water. The following series, for example, was used: 21, 18, 15, 12, 9, 6, and 3 grams per liter of halide, computed as chlorine. Approximate dilutions are prepared and the precise chlorinity later determined by titration with $AgNO_3$. Each of several 100-ml. portions of each dilution is treated with a suitable amount of 0.075N HCl added from a micro-burette permitting a precision of $\pm$ 0.001 ml. Each of the mixtures is then read at 20° C. with brom phenol blue as indicator in a pH bicolorimeter of the Hastings type. The precision of this instrument, provided its optical system is adjusted to give perfectly uniform illumination on both sides, is far beyond the expectations of one accustomed to older pH colorimetric methods. Differences corresponding to less than 0.01 of a pH unit can be recognised and measured by the experienced observer. From the readings, pH values are computed, using 3.98 as the pK_I of brom phenol blue. This "constant" varies, of course, with salinity, but if the same pK_I of the indicator is used throughout, the so-called "salt-error" is automatically eliminated. The value 3.98 is convenient because it is the one established for brom phenol blue at 20° C. in Clark and Lubs' standard buffer solutions. From the pH value and the quantity of acid added are calculated the milliequivalents of H ion neutralized per liter, or the apparent buffer capacity of the carbonate-free water. The several values for each dilution are plotted against the observed pH values, and when this is done for each of the several dilutions, a contour chart involving the three variables, pH, apparent buffer capacity, and chlorinity of the acidified water, is obtained. By interpolation, apparent buffer capacity for any given pH and chlorinity can be read off.

TABLE I

"Apparent" buffer capacity of carbonate-free, approximately neutralized sea water. Milliequivalents of H ion apparently neutralized per liter.

Cl after adding HCl	pH observed after adding HCl											
	4.10	4.05	4.00	3.95	3.90	3.85	3.80	3.75	3.70	3.65	3.60	3.55
gram/l.												
3	*.048*	.055	*.061*	.071	*.080*	.092	*.105*	.121	*.139*	.156	*.179*	.206
4	.058	.065	.075	.084	.095	.107	.123	.139	.158	.177	.203	.232
5	.067	.075	.087	.097	.110	.122	.140	.157	.177	.198	.226	.256
6	*.076*	.085	.098	.108	*.127*	.137	.155	.173	*.196*	.216	.247	.278
7	.083	.093	*.109*	.120	.135	.150	*.171*	.188	.209	.235	*.267*	.299
8	.090	*.101*	.116	*.129*	.146	*.163*	.182	*.204*	.224	*.253*	.285	.319
9	*.096*	.109	.125	.139	*.155*	.174	.194	.215	*.239*	.267	.302	*.339*
10	.103	.116	*.132*	.147	.167	.185	*.205*	.228	.251	.282	*.317*	.354
11	.109	.123	.140	.156	.176	.194	.215	.239	.264	.295	.332	.370
12	*.114*	*.129*	.146	*.162*	*.179*	*.202*	.225	*.250*	*.277*	*.310*	.345	*.383*
13	.120	.135	*.151*	.170	.190	.212	*.236*	.259	.288	.319	*.358*	.398
14	.124	.140	.158	.175	.197	.220	.243	.268	.298	.330	.369	.412
15	*.128*	.145	.164	.181	*.201*	.227	.250	.276	*.307*	.340	.380	*.423*
16	.133	*.150*	.168	*.187*	.210	*.232*	.259	*.283*	.316	*.351*	.390	.435
17	.137	.153	.173	.192	.215	.240	.266	.290	.324	.360	.400	.446
18	*.141*	.157	.178	.196	*.222*	.247	.273	.298	*.333*	.369	.410	*.457*
19	.145	.160	*.184*	.200	.225	.253	*.280*	.304	.340	.378	*.419*	.465
20	*.149*	.164	*.186*	.204	*.229*	.258	*.283*	.309	*.347*	.386	*.429*	*.476*
21	.152	.166	.190	.208	.234	.263	.292	.315	.354	.394	.336	.484

After this method had become standardized six different collections of sea water were used for extended sets of measurements. Their sources were: Woods Hole Harbor, two samples, and one each from Narragansett Bay, Atlantic Ocean near Newport, Atlantic Ocean south of Martha's Vineyard, and Atlantic Ocean near Bermuda. From the resulting six contour charts, the mean value of the apparent buffer capacity was computed for each of 59 points distributed over the ranges of chlorinity and pH values which, when plotted, yield the contour chart shown in Fig. 1. The computed 59 points (in italics), together with others read off from Fig. 1, are given in Table I.

The precision of these values was tested by computation of the probable error of each of the mean values entered upon the contour chart. Of the 59 points 40 had a probable error of the mean not exceeding ± 0.003, 9 of them had a probable error less than ± 0.001 and the general order of the probable error was ± 0.002. In no case

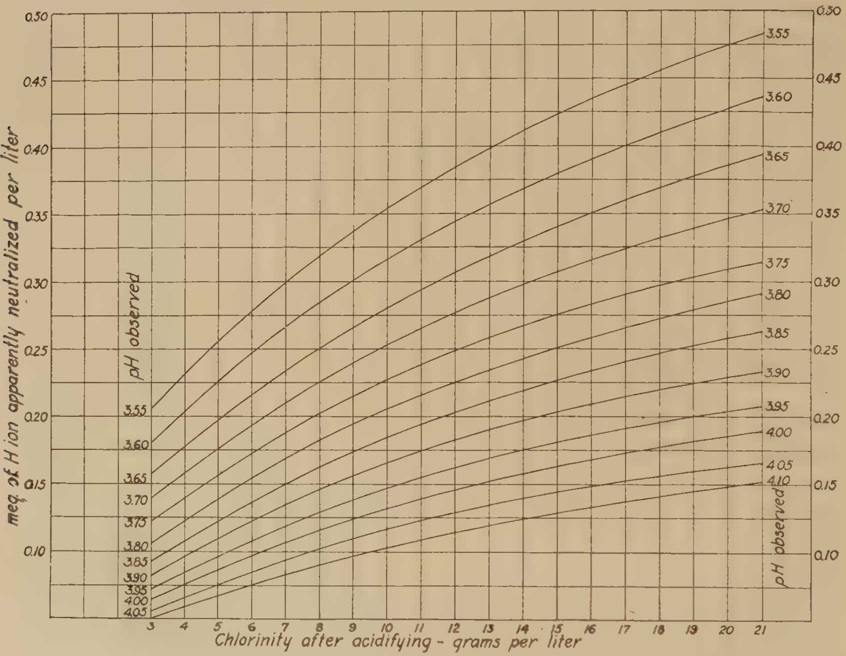


FIG. 1. The apparent buffer capacity of carbonate-free approximately neutralized sea water, used for standard correction values. Above the abscissa equal to the chlorinity of an acidified sea water sample, one selects by interpolation between the isohydric contour lines the pH observed with brom phenol blue as indicator and reads the corresponding ordinate as the correction to be subtracted from the apparent buffer capacity of the sample. These values are computed on the assumption that, for brom phenol blue,

$$pK_t = 3.98 - 0.007(t - 20^\circ \text{ C.}).$$

did the probable error exceed ± 0.006 and the less consistent results were all in a range of pH values more acid than pH 3.70, a part of the chart not recommended for use in more precise measurements, for reasons to be discussed presently.

The final test of the accuracy of these values is their actual use as corrections for measurements of the buffer capacity of sea water. If accurate they should give results on any one sample of sea water, both with and without the addition of distilled water, and at any observed end-point of titration within the chosen pH range, such that the ratio of the buffer capacity (obtained with the use of the carbonate-free correction value) to the chlorinity of the water is constant. This has been found to be the case, in repeated trials, within the limits of observational error. An example of such a test of the method is shown by the results given in Table II.

TABLE II

Titrations of the same sea water sample undiluted and diluted with distilled water

Chloride content	Titration end point	Uncorrected buffer capacity	Correction from contour chart	Corrected buffer capacity	Ratio $\frac{BC}{Cl}$
gram/l.	*pH*	*meq./l.*	*meq./l.*	*meq./l.*	
17.38	3.77	2.394	0.280	2.114	0.1217
17.38	3.79	2.368	0.264	2.104	0.1210
11.98	3.75	1.707	0.247	1.460	0.1219
9.88	3.74	1.439	0.233	1.206	0.1219
5.94	3.87	0.846	0.128	0.718	0.1210
4.05	3.835	0.608	0.115	0.493	0.1217

The selection of an appropriate end-point for the titration of sea water, when brom phenol blue is the indicator, must be a compromise. Greenberg, Moberg and Allen (1932), applying the theory of titration to the problem of methyl orange titration of the total carbonates of sea water, compute the correct end-point as pH 4.35, and use 4.5 in practice. The steepest slope of the titration curve is in the region of pH 4 to 5. This we have found to be the case by the use of the glass electrode described by Taylor and Birnie (1933). This instrument was particularly suitable for the purpose because measurements are taken in a small closed chamber which prevents loss of CO_2. Two titration curves are shown in Fig. 2. They make it obvious that the most sharply defined end-point is at approximately 4.5. Here, however, the effects of carbonic acid are considerable, and loss of CO_2 makes the colorimetric pH estimation very difficult. Indeed, carbonic acid exerts a measurable effect upon the result unless the end-point is distinctly more acid. The true dissociation constant of H_2CO_3, though unknown, is of the general order of pK_a 3.5, according to several observers (Michaelis, 1926). But if the titration is arbitrarily carried to a pH of about 3.4–3.3, that is, beyond the possible effects of carbonic acid, the uncertainty of the colorimetric determination is greatly increased. Readings with brom phenol blue in this range show a comparatively large observational error.

A working compromise between these various objections is reached by selecting the end-point so as to be between pH 3.70 and 4.00, a range within which the titration curve is fairly steep and the measurements can be made with a satisfactory degree of accuracy, while the effects of varying CO_2 tension in the sample are relatively small, affecting the result by an amount less than one per cent of the observed value. The latter fact was ascertained by measuring sea water samples which were equilibrated with laboratory air and then measuring the same samples after bubbling expired air through them. Using 3.98 as the pK_I of brom phenol blue in sea water, as we have proposed above, yields values which are somewhat higher than the *true* pH. In sea water of high salinity at 20° C., the pK_I is of the order of 3.78, as the work of Kolthoff (1930) on KCl solutions has indicated. Precise measurements in sea water of varied salinities and temperatures are desirable. Some preliminary work in this direction has been under-

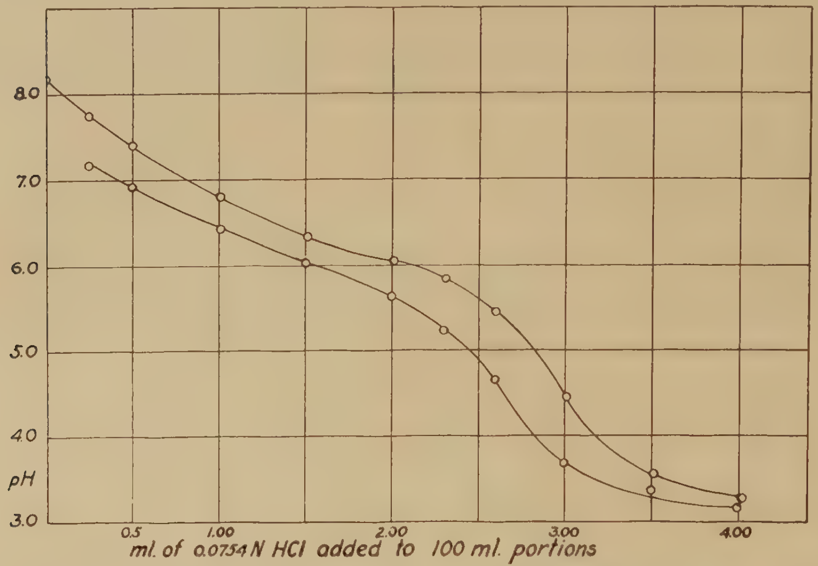


FIG. 2. Sea water titration curves. The pH measurements were taken with a glass electrode in a closed chamber. Each point was obtained with a separate 100-ml. portion of water to which was added the volume of 0.0754N HCl indicated. The pH measurements were made at 30° C.

Upper curve: water from near Newport; chlorinity, 18.10 gram/l.

Lower curve: Narragansett Bay water; chlorinity, 15.21 gram/l.

taken in the laboratory of one of us, using the glass elecrode and the bicolorimeter. It is clear, however, that the end-point chosen for these titrations is sufficiently acid to avoid serious errors due to carbonic acid.

The following procedure is followed in taking the buffer capacity of a sample: It is collected in Pyrex glass, unless measurements can be made at once. Silicates from soda glass can measurably raise the buffer capacity. If it must await analysis for any length of time, it is preserved at a temperature not above 10° C. Fermentation acids can cause low results, in our experience. If any visible organic matter is present the sample is filtered. Protein and perhaps other organic matter raise the buffer capacity. Measurements are taken at or near 20° C. A 100-ml. portion is pipetted into a small Pyrex flask and HCl of about 0.075N strength is added in amount which, as estimated roughly from the salinity of the water, will bring it to the desired acidity as described above. Either preliminary trials or previous experience with water of the locality is necessary to determine the amount. The flask is stoppered and its contents thoroughly mixed and used for pH measurements in the bicolorimeter. The brom phenol blue can give reliable results only when of high purity, which can be ascertained by using it with Clark and Lubs' standard phthalate buffer solutions. Of five different manufacturers' products tested, only two were found to be within the limits of the accuracy of this method. For all the results reported here, brom phenol blue from Hynsonn, Westcott and Dunning was used.

The solution of the indicator must also be approximately isohydric with the solutions to be measured. This condition was sufficiently approximated by adding to the 0.04 per cent indicator solution, as commonly prepared with one molecular equivalent of NaOH, a further amount of 0.01N NaOH equal to one-third of a molecular equivalent. This solution gave the same pH readings in the sea water irrespective of the concentration of indicator. Indicator solutions containing either one or one and one-half molecular equivalents of NaOH failed to do so. An indicator solution containing more than one molecular equivalent of alkali is relatively unstable and should be prepared not more than three days before use.

It is not always feasible to secure temperature control at 20° C. during the pH observation. Although slight variations in temperature do not greatly alter the pH of the acidified sea water, they do affect the dissociation of the indicator to an extent exceeding the limits of accuracy otherwise attainable in this method. As an approximate correction for the temperature effect upon brom phenol blue, we have used the formula, $pK_t = 3.98 - 0.007\ (t - 20°)$, where pK_t is the pK_I at temperature, t. This is taken from a few measurements with the use of the glass electrode and is subject to future correction.

The method of computation of results is shown by the following example. To 100 ml. of Woods Hole Harbor water was added 3.400 ml. of 0.07509N HCl, or 2.553 milliequivalents of H ion per liter of water. The resulting pH was 3.83, or 0.153 meq. of H ion in 1034 ml. of mixture. The apparent uncorrected buffer capacity is 2.553 − 0.153 = 2.400 meq./l. Interpolation from the contour chart of Fig. 1 or from Table I shows that at pH 3.83 and chlorinity of 17.8 grams/l (the value for this mixture), the correction is 0.260 meq./l.

TABLE III

Buffer capacity of surface water from the Cape Cod region

Date	Source of sample	Chloride content	Buffer capacity	Specific buffer capacity
		gram/l.	*meq./l.*	*BC/Cl*
12/19/30............	Near Woods Hole	18.31	2.204	0.1203
"	" " "	18.34	2.220	0.1210
"	" " "	18.28	2.188	0.1198
"	" " "	18.28	2.176	0.1189
"	" " "	18.27	2.171	0.1188
"	" " "	18.17	2.165	0.1192
"	" " "	18.21	2.188	0.1200
7/27/32............	" " "	18.05	2.161	0.1196
"	" " "	18.05	2.138	0.1183
8/30/32............	" " "	18.48	2.181	0.1181
"	" " "	18.48	2.162	0.1170
"	" " "	18.48	2.176	0.1177
9/10/32............	" " "	18.32	2.165	0.1182
9/12/32............	" " "	18.24	2.164	0.1186
"	" " "	18.35	2.159	0.1176
"	" " "	18.24	2.160	0.1182
1/29/33............	" " "	17.90	2.112	0.1181
9/16/32............	Buzzards Bay	18.48	2.223	0.1203
"	" "	18.27	2.191	0.1200
9/22/32............	Cape Cod Bay	18.11	2.183	0.1205
"	" " "	18.02	2.170	0.1203
"	" " "	18.05	2.187	0.1211
9/23/32............	East of Cape Cod	18.22	2.187	0.1200
"	" " " "	18.21	2.189	0.1202
"	" " " "	18.20	2.184	0.1200
"	" " " "	18.19	2.184	0.1201
"	" " " "	18.36	2.200	0.1198
"	" " " "	18.35	2.202	0.1200
8/11/32............	35 miles south of Martha's Vineyard	18.48	2.223	0.1203

The buffer capacity of the sample is, then, 2.400 − 0.260 = 2.140. The $AgNO_3$ titration of this water sample gave Cl 18.32 grams l, so that the ratio BC/Cl = 0.1168. The latter may be called the "specific buffer capacity."

Results of observations on various samples from the Cape Cod region and from parts of the Atlantic Ocean and the Caribbean Sea included in a recent cruise of the "Atlantis," are summarized in Tables

TABLE IV

Buffer capacity of sea water from the Gulf of Maine

Station	Depth	Chloride content	Buffer capacity	Specific buffer capacity
	meters	*gram/l.*	*meq./l.*	*BC/Cl*
1705	0	18.10	2.143	0.1184
	50	18.40	2.200	0.1196
	175	18.74	2.210	0.1179
1706	0	18.10	2.144	0.1185
	50	18.42	2.172	0.1179
	150	18.63	2.182	0.1171
1707	0	18.09	2.211	0.1222
	50	18.25	2.186	0.1198
	125	18.56	2.201	0.1186
1709	0	17.47	2.101	0.1203
	31	17.97	2.130	0.1185
1711	0	17.99	2.161	0.1201
	50	18.16	2.167	0.1193
	175	18.86	2.220	0.1177
1713	0	18.22	2.189	0.1201
	50	18.45	2.207	0.1196
	175	19.17	2.254	0.1176
1717	0	18.08	2.180	0.1206
	50	18.34	2.202	0.1201
	200	19.69	2.290	0.1163
1722	0	18.14	2.104	0.1160
	54	18.43	2.196	0.1192
	114	18.90	2.217	0.1173
	0	18.14	2.173	0.1198
	50	18.445	2.181	0.1182
	250	19.66	2.300	0.1170
1723	0	18.765	2.249	0.1199
	70	18.465	2.215	0.1200

III, IV, V, and VI. (The results in Table VI were obtained by Mr. Homer P. Smith, on board the "Atlantis.") Figure 3 shows the location of the principal stations in the investigation.

The specific buffer capacity, or the ratio of buffer capacity to the

TABLE V

Buffer capacity of Atlantic Ocean water

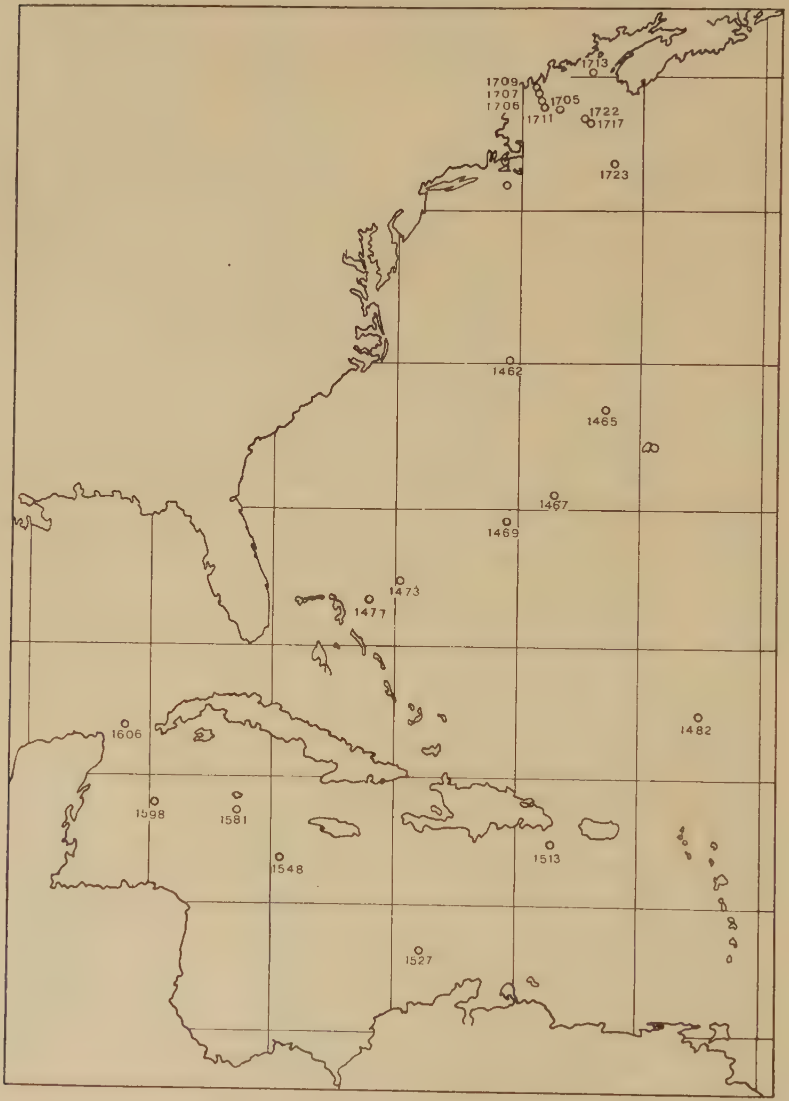
Station	Depth	Chloride content	Buffer capacity	Specific buffer capacity
	meters	*gram/l.*	*meq./l.*	*BC/Cl*
1462	0	20.90	2.420	0.1158
	0	20.90	2.452	0.1172
	100	20.90	2.450	0.1171
	2000	19.95	2.393	0.1199
	2000	19.95	2.371	0.1188
St. Georges Harbor, Bermuda	0	21.00	2.403	0.1144
1465	0	21.15	2.438	0.1152
	100	20.17	2.363	0.1171
1467	0	21.20	2.373	0.1120
	100	21.20	2.419	0.1140
	4500	20.17	2.378	0.1179
1469	0	21.20	2.402	0.1134
	100	21.15	2.419	0.1143
	600	20.83	2.386	0.1145
	800	20.45	2.380	0.1164
	1200	20.30	2.347	0.1157
	2000	20.22	2.338	0.1156
	3000	20.20	2.346	0.1161
	4000	20.20	2.346	0.1161
1473	0	21.22	2.384	0.1123
	100	21.20	2.374	0.1122
	500	21.07	2.367	0.1123
	1000	20.35	2.328	0.1144
	1600	20.20	2.317	0.1147
	2000	20.20	2.329	0.1153
	2500	20.20	2.329	0.1153
	3000	20.20	2.317	0.1147
1477	0	20.93	2.356	0.1125
	100	21.17	2.385	0.1126
	500	21.03	2.387	0.1135
	1000	20.27	2.319	0.1144
	1400	20.25	2.319	0.1145
	2000	20.25	2.295	0.1133
	3000	20.20	2.295	0.1136

TABLE VI

Buffer capacity of water from the Caribbean Sea

Station	Depth	Chloride content	Buffer capacity	Specific buffer capacity
	meters	*gram/l.*	*meq./l.*	*BC/Cl*
1482	100	20.855	2.406	0.1154
	500	20.375	2.367	0.1162
	1000	19.825	2.384	0.1203
	2000	19.845	2.384	0.1201
	3000	19.785	2.335	0.1180
1513	100	20.495	2.296	0.1120
	500	19.98	2.270	0.1136
	1000	19.745	2.245	0.1137
	2000	19.81	2.271	0.1146
	3000	19.81	2.271	0.1146
1527	0	20.65	2.355	0.1140
	100	20.755	2.389	0.1151
	500	19.77	2.310	0.1168
	1000	19.785	2.322	0.1174
	2000	19.82	2.335	0.1178
	3000	19.83	2.347	0.1184
1548	0	20.35	2.319	0.1140
	100	20.48	2.355	0.1150
	500	19.95	2.310	0.1158
	1000	19.74	2.298	0.1164
1581	0	20.425	2.355	0.1153
	100	20.435	2.368	0.1159
	500	20.07	2.309	0.1150
	1000	19.685	2.311	0.1174
	2000	19.755	2.298	0.1163
	3000	19.775	2.298	0.1162
	4000	19.76	2.258	0.1143
	5000	19.775	2.258	0.1142
	6000	19.775	2.229	0.1127
1598	0	20.38	2.391	0.1173
	100	20.36	2.381	0.1169
	500	20.00	2.346	0.1173
	1000	19.725	2.323	0.1178
	2000	19.77	2.310	0.1168
	3000	19.775	2.327	0.1177
			2.310	0.1168
1606	0	20.415	2.367	0.1159
	100	20.40	2.344	0.1149
	500	20.07	2.296	0.1144

chlorinity, is in general lower than that reported by Thompson and Bonnar for Pacific water of the San Juan Archipelago and nearby regions. That this difference is not due merely to variations in method is indicated by the results of measurements made by one of us on 48 samples from various bays in the Puget Sound region. These measurements were made by the same method as that used in this report and the same correction values for salt effects were applied. All the re-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1527

FIG. 3. Location of the principal stations.

sults fell within the range of specific buffer capacities reported by Thompson and Bonnar. It is hoped that the titratable alkalinity of Atlantic and Pacific waters can be more adequately compared in the near future. It would also be of interest to compare the ratios we have already found with those obtained in other parts of the world, but it is not yet certain in our opinion that results obtained by different methods are fairly comparable. So far as we are aware, the report of Thompson and Bonnar is the only one based upon the use of this method.

A comparison of our data with those of Wattenberg (1925–27), in the course of his extensive work in the South Atlantic on the "Meteor" expedition, shows very fair agreement. His values for the ratio of alkalinity to chloride fall mostly between 0.120 and 0.125, but using chloride per kilogram as a basis. When this is changed to chloride per liter (lowering the ratio about 2 per cent) very close agreement results with our average value of 0.119 + for water near Cape Cod.

Two general tendencies are to be seen in the above data: first, a lower specific buffer capacity in waters to the south than in those from the neighborhood of Cape Cod and the Gulf of Maine. This difference is especially noticeable in the western part of the Sargasso Sea and in the greater portion of the Caribbean. A full discussion of the significance of these differences must await further data, which we are endeavoring to obtain.

Were these variations due simply to a difference between coastal and mid-ocean water we should expect the results in the Caribbean to be similar to those in the Gulf of Maine, but it is precisely here that the difference is most marked. Wattenberg's values for "specific alkalinity" in the South Atlantic show a similar, but less pronounced, variation with respect to latitude. Averaging his results from stations in the neighborhood of 40° – 50° S. on the one hand, and those from stations 10° – 20° S. on the other, shows an increase of about 2 per cent, at corresponding depths, in the higher latitudes. A similar analysis of our more limited data reveals a difference of 4–5 per cent.

Second, there is a variation in specific buffer capacity with depth; in general, and especially at deep-sea stations, an *increase* with depth. (Wattenberg found the specific alkalinity to behave in the same manner.) In two widely separated areas, however, this variation is reversed. Both in the Gulf of Maine and in the western part of the Caribbean the buffer capacity consistently *decreases* with increasing depth. We are not yet in a position to discuss the full significance of these facts, beyond suggesting that mass movements of the water are concerned, as well as possible variations in the character of the land drainage.

It may be pointed out that the lowering of the specific buffer capacity resulting from the mixing of mid-ocean and coastal water has been apparent in several of our observations. For example, during a prolonged and violent easterly storm in April samples taken at Woods Hole had a higher chlorinity and correspondingly a lower ratio than any we had ever observed there, between 0.1138 and 0.1149.

In addition to the work here reported, many measurements have been made on samples from the Narragansett Bay region. Here, however, the buffer capacities are variable and of a different order from those observed in ocean water. The effects of land drainage, rainfall, tidal and other currents, etc., involved in a nearly land-locked body of water such as Narragansett Bay, are sufficiently complex to deserve special treatment, which will be undertaken in a later report.

SUMMARY

1. The method of Thompson and Bonnar for measuring the buffer capacity of sea water has been modified by the introduction of the use of standard correction values to make allowance for the effects of salts upon hydrogen ion activity and upon the dissociation of brom phenol blue. These values are reported in tabulated and graphic form.

2. Various sources of error in the method are discussed and corresponding refinements of technique suggested.

3. The specific buffer capacity of Atlantic waters according to these measurements is lower than that of Pacific waters, so far as yet investigated.

4. Lower specific buffer capacities were found in the Atlantic Ocean to the southward and in the Caribbean Sea than in the neighborhood of Cape Cod and the Gulf of Maine.

5. In general, an increase in buffer capacity with respect to depth was observed, but in the Gulf of Maine and the western part of the Caribbean this relation was distinctly reversed.

6. The effects of mass movements of ocean water upon the buffer capacity appear to be traceable.

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Contribution No. 33

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DIURNAL MIGRATION OF PLANKTON IN THE GULF OF MAINE AND ITS CORRELATION WITH CHANGES IN SUBMARINE IRRADIATION

DIURNAL MIGRATION OF PLANKTON IN THE GULF OF MAINE AND ITS CORRELATION WITH CHANGES IN SUBMARINE IRRADIATION

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The investigations of Russell (1926, 1927, 1931), Kikuchi (1930), Worthington (1931), and Southern and Gardiner (1932) reveal the magnitude and the importance of the diurnal migrations of zoöplankton. The observations indicate that light is probably the most important factor controlling these movements, although it is uncertain in just what way the organisms respond to changes in the intensity of light. There is likewise considerable doubt in regard to the parts played by other factors. The present investigation was undertaken to study in more accurate detail the migrations of plankton organisms —particularly copepods—and to test the theories regarding the actual rôle of light.

In previous investigations the intensity of the light in the water was not measured at the same time that the plankton hauls were made.[2] Usually the submarine irradiation was assumed to be proportional to the light present at the surface as indicated by the time of day and the condition of the weather. Russell (1931) has estimated values for the irradiation at various depths on the basis of measurements made on other occasions (Poole and Atkins, 1929). In the present investigation it was therefore proposed to record the irradiation on deck throughout daylight hours when plankton hauls were in progress and to measure the percentage of surface light penetrating to various depths in the water at suitable periods during the day. The intensity of light to which the organisms are subjected at different levels would thus be precisely known.

To improve the accuracy of the determination of the vertical distribution of the animals, five nets were constructed which could be towed simultaneously at different depths and opened and closed in position. I am indebted to Mr. C. O'D. Iselin of the Woods Hole

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[1] Contribution No. 33.

[2] Russell, in collaboration with Atkins, has recently made such observations, the results of which are as yet unreported (see Atkins and Poole, 1933).

Oceanographic Institution for the main ideas involved in the construction of these nets and for the actual design of the fittings. By using these at intervals during the day and night, we hoped to obtain significant information in regard to the vertical migrations taking place.

The observations on the diurnal migration of plankton reported in this paper were made from the "Atlantis," the research vessel of the Woods Hole Oceanographic Institution, at Station 1053 (in August, 1931) and at Stations 1285, 1286, and 1287 (in July, 1932)—all in the Gulf of Maine. The details of the measurement of the penetration of daylight are presented in a separate paper (Clarke, 1933).

Method of Making Hauls

The five closing nets were clamped at intervals on the main dredging cable and towed horizontally through the water, thus sampling the plankton at five different depths in one operation. The original nets were made of silk but these were abandoned because they were too easily injured by washing against the cable—a circumstance unavoidable in the method used. The nets employed in 1932 were made of heavy cotton "scrim" (10 strands/cm.) and proved very satisfactory. They are 3 meters long, 75 cm. in diameter at the mouth, 11 cm. in diameter at the bottom, and provided with a pursing rope 1 meter from the mouth.

The canvas sleeve at the bottom of each net fits snugly over the rim of a light brass bucket (11 cm. in diameter and 17.5 cm. deep), similar to that described by Kemp and Hardy (1929). A brass band is slipped over the canvas and clamped tight by means of a bolt with winged nut. The bucket is thus secured firmly to the net and can be attached or removed quickly. An opening on one side covered with netting of monel metal (40 strands/cm.) allows about two-thirds of the water in the bucket to drain off so that the catch can be poured directly into a 1/2 liter bottle without further straining.

The main dredging cable (1.27 cm. in diameter) to which the five nets are attached, travels from the winch forward along the deck and then outboard to the end of a boom located amidships. The cable is kept as nearly vertical as possible by a stream-lined weight (680 kilograms in air) fastened to the lower end. During this investigation the angle which the cable made with the vertical never averaged more than 23° for each towing period. The work of attaching the nets to the cable is performed at the end of a platform rigged out from the side of the ship.

Each net is laced to its frame, consisting of a brass hoop, and this is attached directly to the cable by means of clamps, no bridle being

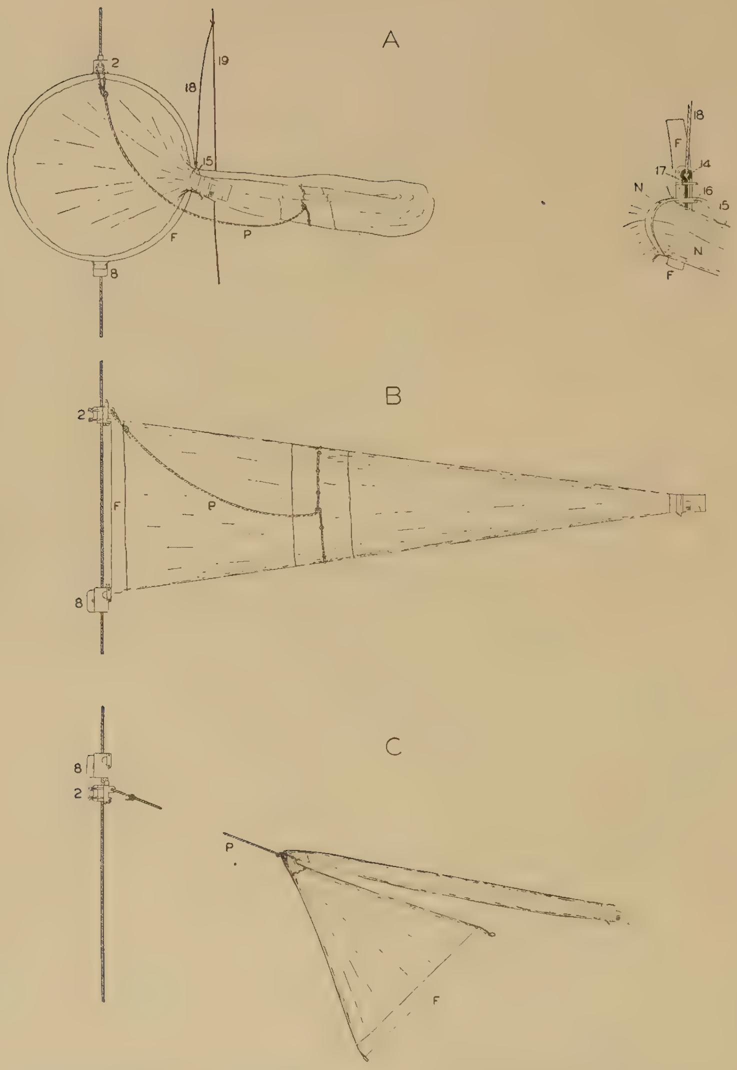

FIG. 1. Diagrams to show the method of using the nets.

A. Net closed and arranged for lowering preparatory to making haul. Net frame moves edgewise through the water.

Inset: Enlargement of edge of net frame showing in detail the strap and pin device.

B. Net open and fishing. The opening is unobstructed except for the single cable passing across it.

C. Net closed and ready to be hoisted aboard. The net frame has been cast off and the net is drawn shut on the pursing rope. Note the "messenger" clamp (8) which has dropped from the net above on to the trigger of the upper clamp (2), thus releasing the closing mechanism.

14. Pin.
15. Strap.
16. Brackets.
17. Thread stopper.
18. Cord attaching pin to (19).
19. Hand-line to which all the pins are attached.

F. Frame.
N. Netting.
P. Pursing rope.

used (see Fig. 1, *B*). The top clamp is composed of two separate parts, as shown in Fig. 2, *A*. The split lug (1), which is cast to fit the lay of the cable, is first rigidly fixed in position by tightening the four screws. Since the lug is small enough to pass through the sheaves, this operation may be performed on deck and the cable then lowered away until the lug reaches a position about two meters above the water. The second part (2), of the upper clamp, is next closed around the lug as shown in *B* (Fig. 2). This fitting swivels freely around the lug. The upper edge of the net frame is provided with a flanged plate (3) which is next secured in the slot (4) of the fitting. This is accomplished by depressing against an internal spring the trigger end (5) of the U-shaped bolt (6). The latch end (7) of the bolt is thus withdrawn from the slot (4), and plate (3) is allowed to slip in. When pressure on the trigger

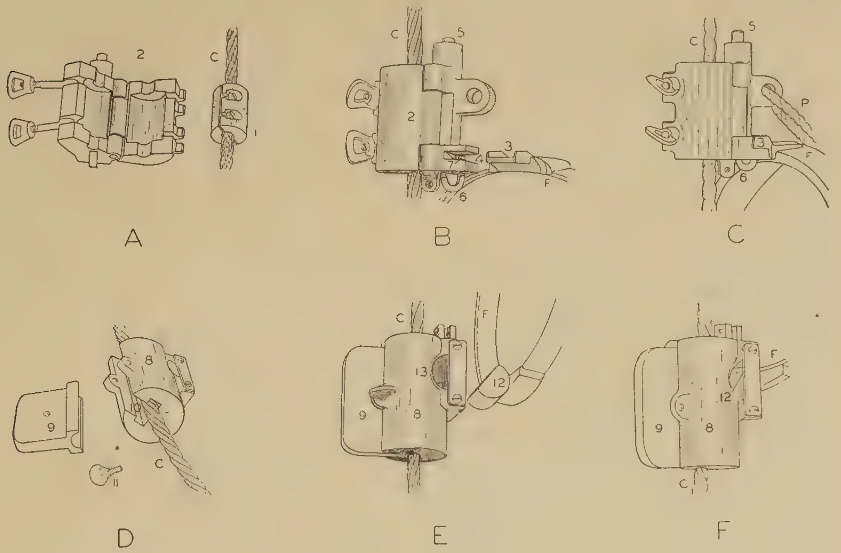

FIG. 2. Method of attaching closing net to cable. For explanation see text.

A, *B*, and *C*—upper clamp and top of net frame.
D, *E*, and *F*—lower clamp and bottom of net frame.

1. Split lug secured to cable.
2. Outside part of upper clamp which encloses (1).
3. Flanged plate at top of frame.
4. Slot into which (3) fits.
5. Trigger end of (6).
6. U-shaped bolt.
7. Latch end of (6).
8. Cylindrical part of lower clamp.
9. Key which slips into (10).
10. Groove in (8).
11. Thumbscrew.
12. Projection at bottom of frame.
13. Socket into which (12) fits.

C. Main dredging cable.
F. Net frame.
P. Pursing rope.

is released, the latch (7) snaps back, passing through a hole in plate (3) and locking it into position (*C*, Fig. 2). The pursing rope (*P*) is made fast to the eye provided in this fitting.

The next step is placing the lower clamp in position as shown in *D* (Fig. 2). The cylindrical part (8) is slipped over the cable and the key (9) is slid up into the groove (10) where it is held by the thumbscrew (11). Before this is done, however, the clamp must be attached to the lower edge of the net frame. This is provided with a projection (12) in the shape of a 3/4-cylinder. The projection can slip into or out of the similarly-shaped socket (13), which is open in front, except when the net frame and the longitudinal axis of the fitting (8) are held parallel. Accordingly, in attaching the lower clamp the fitting (8) is first tilted until the projection (12) slips into the socket (13). It is then straightened up, thus locking the projection in, and the key (9) is slid into place, thus securing clamp and frame to the cable (*F*, Fig. 2). The whole clamp is free to swivel around the cable. Since the upper fitting (2) can also turn, the net frame is free to rotate horizontally although it cannot slide up or down on the cable.

To prevent the net from fishing on the way down (and while the other nets are being attached), it is closed at the mouth by drawing the netting across the opening to one edge of the frame (see Fig. 1, *A*). The netting is held here by a strap, the lower end of which is permanently fastened to the frame. The upper end is provided with a hole (see inset, Fig. 1, *A*) through which the brass pin (14) passes, thus securing the strap (15) between the two brackets (16). A thread stopper (17) prevents the pin from coming out fortuitously. The tail of the net is doubled back and placed under the strap, because if left free, it would lash about and become fouled. The pin (14) is attached to the hand-line (19) by the short piece of cord (18). The pins of all the nets are similarly fastened in succession to this line.

The net is now ready for lowering and the ship is put under way at the lowest possible speed. As soon as the net is in the water, it is swung around by the loose netting trailing out behind. The net frame accordingly tows edgewise through the water and does not fish. Meanwhile the next lug has been attached to the cable at the proper distance inboard. This is run out to the position over the water where the next net is attached exactly as before.

When all the nets have been attached to the cable and the whole string lowered to the desired depth, the ship is accelerated to the regular towing speed (see below). The nets are then opened simultaneously by hauling on the handline (19). When all the pins are pulled free, the line with the five pins attached is taken in on deck. Instantly the pressure of the water causes the nets to open out and to fish as shown in *B*, Fig. 1.

At the end of the towing period the nets are all closed before they are raised to the surface. This is accomplished as follows. A "messenger" (an extra lower clamp) is placed on the cable and allowed to slide down it. When this reaches the topmost net, it strikes the trigger (5). The latch end (7) of the bolt is thereby forced downward and the plate (3) is pulled free by the horizontal pressure of the water on the net. Once the frame of the net tilts backward in this way, the projection (12) slips out of its socket and the frame becomes detached from the lower clamp. Since there is now no vertical support for the lower clamp, it immediately slides down the cable and acts as the "messenger" to release the next net. In quick succession the frames of all five nets are thus cast off from the cable, leaving the nets hanging from the upper clamps by their pursing ropes only. The drag of the net through the water causes these to draw tight and to effectively close the nets as is shown in *C*, Fig. 1. Finally, the nets are raised and taken on deck in the reverse order in which they were sent down. With a little practice this whole operation, including the 10-minute tow, can be completed and the nets made ready to be sent down again in about three-quarters of an hour.

The actual depths at which the five nets were towed at the different stations are given in the tables. In general, the nets were spaced in such a way as to sample the upper 50 meters of water to the best advantage. During the middle of the day, when the plankton was expected to occur at its maximum depth, the series of hauls was repeated at a lower level, the five nets then sampling the water between 50 meters and 100 meters. On one day this was done at other hours as well. The angle which the cable made with the vertical was measured in the usual way at the beginning, middle, and end of each towing period and was found to vary ordinarily between 10° and 20°. In those few cases in which the angle during this period averaged more than 20°, the depths of all the nets were corrected by multiplying by the cosine of the average angle.

The towing period was 10 minutes in all cases. The speed of the ship was carefully determined at least three times during the tow by measuring with a stop-watch the time required for a section of the ship's deck 15.2 meters long to pass a chip of wood tossed into the water. By adjusting the speed of the ship from time to time, the average speed for each tow was kept within the limits of 1.0 to 1.8 knots. At a speed of 1.25 knots, the average for all the hauls, the nets would be drawn through 366 meters of water during the 10-minute period, thus theoretically straining 162 cubic meters. Although the speed of towing varied slightly, it is important to note that it was necessarily the same for all five nets on the line. Differences in the number of animals caught at different depths at the same time, therefore, could not be due to variation in the towing speed.

The hours at which the series of plankton hauls were made are indicated in the tables. At Station 1053 five series were made during the morning. Further observations were unfortunately prevented by bad weather. During the 24-hour period comprising Stations 1285 and 1286, four series were made and each repeated at the lower level. At Station 1287 nineteen series (2 repeated) were made during a continuous 48-hour period. In general the series were made at more frequent intervals of time during the early morning and in the evening because the plankton animals are known to migrate most rapidly during those periods.

Method of Estimating Catches

The plankton catches were preserved in 10 per cent formalin and transported to the laboratory where the numbers of the different kinds of organisms were estimated by the following sampling method. Each catch was poured into a large cylindrical battery jar graduated in liters. Water was then added until a suitable dilution was attained, the total volume varying, according to the size of the catch, from 1 liter to 10 liters. The liquid was then thoroughly stirred by an electrically driven propeller. As soon as the plankton organisms became evenly distributed, four samples were taken with a dipper of 29-cc. capacity (consisting of a small porcelain crucible provided with a long handle).

Each sample was poured out upon a ruled counting slide (3.5 × 7.2 cm.) and placed under the binocular microscope. By the use of a mechanical stage the whole sample can be rapidly passed in review. Each individual was examined in turn and its species, sex, and age group recorded. The analysis was limited to those forms which occur in significant numbers. If the first two samples did not vary by more than 10 per cent from their average, no more samples were counted. The samples often gave counts closer than this, indicating that the method used is sufficiently accurate for the purpose (see below). If agreement was not good, the third and, if necessary, the fourth sample was examined. In the case of very large catches it was sometimes necessary to sub-sample the original samples. The same method was used but more samples were counted to insure the same degree of accuracy.

Sources of Error

One of the most serious sources of error occurring in investigations of this type is the irregularity in the horizontal distribution (*i.e.*, "swarming") of the plankton. The present method, employing five nets towed simultaneously on a single cable, has certain advantages over previous methods in reducing the magnitude of this error. In the first place, the five hauls of a single series contain no error at all from this source when compared among themselves since they were made at the same time and place. But since the successive series of hauls are not made in exactly the same body of water (due to the drift of the ship), "swarming" would introduce an error for comparisons between the hauls in different series. However, since the nets are towed a considerable distance horizontally, this error is less than would result from the use of a single net hauled vertically (cf. Southern and Gardiner, 1932; and Worthington, 1931). This method is likewise an improvement over those in which a single horizontal net has been towed at different depths in succession. In such cases the individual hauls have been made in different bodies of water, thus introducing the possibility of an error within each series (cf. Russell, 1925).

The use of five nets at once has eliminated another error previously encountered. This was due to the relatively long interval between the times when the first haul and the last haul of a series were made and the consequent possibility that the distribution of the plankton had changed meanwhile. Furthermore, the present method is sufficiently quick to enable complete observations to be repeated at intervals of less than an hour.

In view of the precautions taken, as described above, the errors due to differences in speed and duration of towing (and hence in amount of water strained) and in the depths of the nets are probably negligible. Moreover, it is important to note that the speed and duration are of necessity the same for all the hauls in the same series. The distance between the nets on the cable remained the same, of course, throughout each towing period, and could not have varied by more than a fraction of a meter in different series.

The use of closing nets which are allowed to fish only at their proper depths increases the accuracy of the observations over that possible when open nets are used. However, since the nets are towed horizontally, the layers of water between the nets are not sampled and in the present case there was a considerable body of water beneath the lowest net the population of which was not investigated at all. (Bottom at 256 meters at Stations 1285 and 1286, 146 meters at Station 1287.) Since the investigation was undertaken primarily to test the effect of light, attention was centered on the surface layers. Even at the brightest time of day, light sufficiently intense to be measured did not penetrate to a depth greater than about 50 meters. In the case of the organisms for which we have the most accurate information (*Metridia lucens* adult female), the zone of maximum abundance did not sink below the 54-meter depth at the hour when it would be expected to occur at its lowest for the day (see Figs. 3 and 5). It seems probable that the picture of the vertical distribution of this form would not be materially altered if information on the population of the water layers between the levels of the five nets could have been obtained.

Certain other sources of error common to all similar investigations should be mentioned, the importance of which has long been in question. First of all, there is the possibility that because of clogging the nets might vary in the amount of water actually strained. No satisfactory method has yet been devised to remove this source of error. In the present work the precaution of thoroughly washing out the nets after each haul was always taken. Probably more serious is the possibility that the animals escape the net to varying degrees on different occasions. The speed of towing and the size of the mouth of the net (cf. Gardiner, 1933) are important factors in this connection, but whatever their effects may have been in the present investigation, they were the same for all the hauls. Since our nets have no bridles, the water is stirred up to a minimum extent in front of their openings. Whatever ability the plankton animals may have to see the net and to avoid it to a greater extent in bright light than in weak light or darkness would tend to cause differences in the numbers caught (1) between surface catches made during the day and those made at night and (2) between daytime catches made at the surface and those made at depths where the light is greatly reduced. This question has been thoroughly discussed by Russell (1928), Worthington (1931), and Southern and Gardiner (1932). It is useless to compare day and night totals in the present case (as previously done), since no attempt was made to investigate the bottom layers of water.

Finally, the sources of error in the estimation of the catch must be examined. The method used is comparable to those employed by Russell (1925) and Worthington (1931), which appear to be satisfactory. In an extensive investigation of this matter Gardiner (1931) found that the enumeration error when using a similar method (the Hensen Stempel-pipette) was insufficient to account for the variation of the catch. The error resulting from sampling is undoubtedly large, but in view of the huge variation in numbers of animals caught it is probably *relatively* small, if not negligible.

It seems permissible to conclude that the methods used in this investigation are at least as accurate as those previously employed and that the results may be relied upon. The modifications which have been introduced—particularly the use of five simultaneous closing nets —allow special dependence to be placed upon the differences found among the hauls within a single series.

Observations

General Vertical Distribution

The following three species of copepods comprise the bulk of the catches made: *Metridia lucens* (Boeck), *Centropages typicus* (Krøyer), and *Calanus finmarchicus* (Gunner). In most of the hauls made at Stations 1285 and 1286 and in a few of those at Station 1287, quite large quantities of siphonophore fragments were taken as well. A general view of the numbers of each species of copepod present and of the relative depth at which each lives may be obtained from Fig. 3.

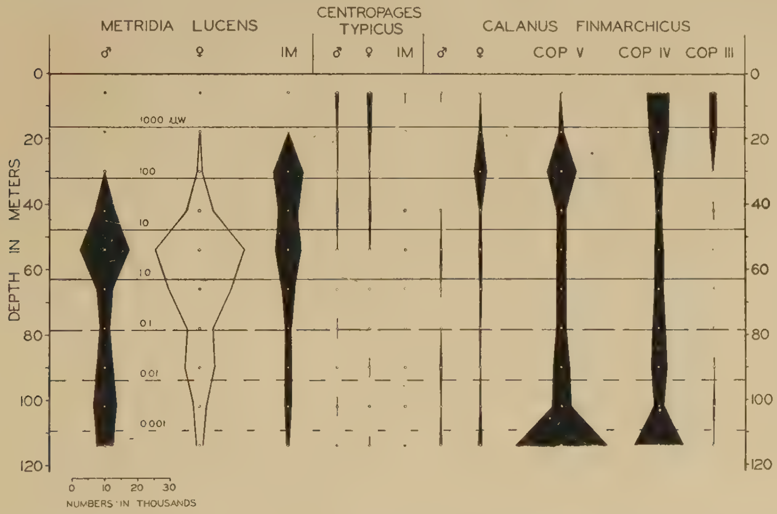

FIG. 3. General view of the vertical distribution and the relative numbers of the three species of copepods studied. The diagram for the adult females of *Metridia* is plotted on one-half the scale of the others. The depth at which light intensities of 1000 microwatts, 100 microwatts, etc., occurred are indicated by the horizontal lines. (One microwatt may be taken as approximately equal to three meter-candles as used by Poole and Atkins, 1929.) The upper five hauls (A244 to A248) were made at 1439 o'clock, the lower five (Nos. A249 to A253) at 1515 o'clock on July 11, 1932, at Station 1287.

The diagrams, constructed in the usual way (cf. Russell, 1925), represent the copepod population down to 114 meters at Station 1287 early in the afternoon of July 11, 1932. This particular series has been selected as being the most complete and representative. The numerical data for all the series are presented in Tables I, II, and III.

TABLE I

Station 1053, August 24, 1931, Lat. 43°27′N., Long. 67°39′W. Sea rough, sky overcast. Sunrise at 0511. Numbers of copepods in thousands. x = less than 500.

Haul No.	Depth	Local Sun Time †	Irradiation at Surface	Irradiation at Haul Depth	Irradiation 30 min. before	Centropages typicus			Calanus finmarchicus				
						Adult ♂	Adult ♀	Immature	Adult ♂	Adult ♀	Cop. V	Cop. IV	Cop. III
	meters		*microwatts*	*microwatts*	*per cent*								
A55	4.5	0456	19	9.3	No light	282	223	648	0	0	0	0	0
56	14.	"	"	2.7	" "	12	26	4	0	x	x	1	2
57	23.	"	"	1.0	" "	2	9	1	x	11	92	52	27
58	32.	"	"	—	" "	x	2	1	x	15	88	34	3
59	46.	"	"	—	" "	1	2	1	x	6	15	2	0
60	4.5	0602	970	480.	49	16	25	308	0	0	0	0	0
61	14.	"	"	140.	"	103	107	150	0	0	0	0	0
62	23.	"	"	50.	"	1	x	x	x	18	77	53	36
63	32.	"	"	9.1	"	x	x	0	1	13	79	61	11
64	46.	"	"	2.0	"	x	1	x	0	2	11	4	0
65	4.5	0656	1930	950.	57	4	10	153	0	0	0	0	0
66	14.	"	"	270.	"	94	77	69	x	x	x	x	x
67	23.	"	"	100.	"	x	1	x	0	2	26	28	12
68	32.	"	"	18.	"	x	x	x	x	3	32	27	6
69	46.	"	"	4.1	"	x	1	0	x	2	21	13	x

TABLE I—*Continued*

Haul No.	Depth	Local Sun Time †	Irradiation at Surface	Irradiation at Haul Depth	Irradiation 30 min. before	Centropages typicus			Calanus finmarchicus				
						Adult ♂	Adult ♀	Immature	Adult ♂	Adult ♀	Cop. V	Cop. IV	Cop. III
	meters		*microwatts*	*microwatts*	*per cent*								
70	4.5	0807	5000	2450.	64	9	11	296	0	0	0	0	0
71	14.	"	"	700.	"	1	1	35	0	0	0	0	0
72	23.	"	"	260.	"	26	21	84	0	0	0	0	0
73	32.	"	"	47.	"	2	4	1	0	*x*	*x*	*x*	*x*
74	46.	"	"	10.5	"	*x*	*x*	*x*	*x*	*x*	*x*	1	*x*
75	4.5	1227	7100	3500.	150	9	14	360	0	0	*x*	0	0
76	14.	"	"	990.	"	7	12	115	0	0	0	0	0
77	23.	"	"	370.	"	194	220	400	0	0	0	*x*	26
78	32.	"	"	66.	"	2	3	2	0	*x*	11	25	23
79	46.	"	"	15.	"	*x*	1	*x*	0	2	5	5	1
80	64.	1330	7500	3.4	76	0	*x*	*x*	0	2	8	5	*x*
81	83	"	"	—	"	1*	2*	1*	*x*	1	3	1	*x*
82	101	"	"	—	"	*x**	*x**	*x**	*x*	*x*	2	1	*x*
83	120	"	"	—	"	*x**	*x**	1*	0	*x*	2	1	0

* Mostly cast shells.

† 24-hour scale. The time indicated is the middle of the 10-minute towing period.

TABLE II

Hauls A145–A174, Station 1285, July 9, 1932, Lat. 42°34′N., Long. 69°37′W. Sea, slight; sky, clear. Sunrise 0431. Sunset 1939.

Hauls A175–A183, Station 1286, July 10, 1932, Lat. 42°30′N., Long. 69°37′W. Sea, slight; sky, few clouds. Sunrise 0431. Sunset 1938.

Numbers of copepods in hundreds. x = less than 50.

Haul No.	Depth	Local sun time	Irrad. at surface	Irrad. at haul depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus				Metridia lucens						
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Ad. ♂	Ad. ♀	Cop. V ♂	Cop. V ♀	Cop. IV ♂	Cop. IV ♀	Cop. III
	m.		μW	μW	per cent														
A145	5	1119	14,900	6900.	100	x	5	x	0	9	9	9	0	0	x	0	0	0	0
146	15	"	"	1500.	"	0	0	0	0	2	13	6	0	0	2	2	0	0	0
147	25	"	"	330.	"	0	0	0	x	7	15	5	0	0	x	x	0	0	0
148	35	"	"	46.	"	0	0	0	0	2	20	14	x	x	3	10	x	x	0
149	45	"	"	13.	"	0	0	0	0	4	29	19	x	x	1	4	x	1	x
150	55	1230	16,200	3.7	90	0	0	0	x	2	18	19	1	x	3	6	x	1	0
151	65	"	"	—	"	0	0	0	0	3	23	26	1	1	4	14	x	2	0
152	75	"	"	—	"	0	0	0	0	3	43	65	3	1	4	14	x	1	0
153	85	"	"	—	"	0	0	0	0	7	58	84	8	2	7	26	3	6	0
154	95	"	"	—	"	0	0	0	0	3	96	119	9	3	5	22	0	x	0
155	5	1812	2,700	950.	180	4	15	1	0	2	2	3	0	0	x	0	0	0	0
156	15	"	"	240.	"	1	5	x	0	2	4	2	0	0	x	x	0	0	0
157	25	"	"	110.	"	x	3	0	0	2	10	6	x	x	2	6	0	0	0
158	35	"	"	12.	"	x	1	0	0	x	1	3	x	x	1	4	0	x	0
159	45	"	"	2.7	"	x	x	0	0	x	5	8	x	x	2	5	x	x	0
160	55	1851	1,500	—	"	x	x	0	0	2	35	34	6	7	1	12	0	x	0
161	65	"	"	—	"	0	x	0	0	4	51	58	7	5	x	5	0	x	0
162	75	"	"	—	"	0	0	0	x	3	53	64	6	6	2	6	0	0	0
163	85	"	"	—	"	0	0	0	0	3	36	43	3	1	1	3	0	0	0
164	95	"	"	—	"	2	4	0	0	6	48	37	1	7	3	6	0	0	0

TABLE II—*Continued*

Haul No.	Depth	Local sun time	Irrad. at surface	Irrad. at haul depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus				Metridia lucens						
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Ad. ♂	Ad. ♀	Cop. V ♂	Cop. V ♀	Cop. IV ♂	Cop. IV ♀	Cop. III
	m.		*μ W*	*μ W*	*per cent*														
165	5	2123	No light	No light	No light	3	17	1	0	2	5	3	0	2	*x*	3	0	0	0
166	15	"	" "	" "	" "	*x*	2	*x*	0	1	2	*x*	0	1	*x*	4	0	0	0
167	25	"	" "	" "	" "	*x*	0	0	0	3	26	14	1	16	1	8	0	0	0
168	35	"	" "	" "	" "	0	*x*	0	0	3	30	41	2	11	1	6	0	*x*	0
169	45	"	" "	" "	" "	0	*x*	0	*x*	3	29	21	*x*	18	0	6	0	*x*	0
170	55	2214	" "	" "	" "	0	0	0	0	6	42	44	4	72	2	7	0	0	0
171	65	"	" "	" "	" "	0	0	0	0	5	53	58	3	76	3	14	*x*	1	0
172	75	"	" "	" "	" "	0	0	0	0	5	53	68	5	75	5	13	0	*x*	0
173	85	"	" "	" "	" "	0	*x*	0	0	3	40	54	7	58	3	15	0	*x*	0
174	95	"	" "	" "	" "	0	0	0	1	4	40	49	4	76	2	16	*x*	*x*	0
175	5	0552	2,180	1200.	48	8	28	1	0	2	3	3	0	*x*	0	*x*	0	0	0
176	15	"	"	350.	"	*x*	*x*	0	*x*	6	11	7	*x*	*x*	4	8	0	0	0
177	25	"	"	100.	"	*x*	0	0	0	3	21	29	3	1	3	8	*x*	*x*	0
178	35	"	"	21.	"	*x*	*x*	0	0	2	12	25	2	2	2	7	0	*x*	0
179	45	"	"	4.4	"	*x*	*x*	0	0	2	19	43	2	3	1	5	0	*x*	0
180	55	0631	3,950	0.5	63	0	0	0	0	5	41	73	6	1	3	9	0	0	0
181	65	"	"	—	"	0	0	0	0	9	41	85	9	1	2	10	0	0	0
182	75	"	"	—	"	0	0	0	*x*	2	11	19	5	*x*	1	6	0	*x*	0
183	85	"	"	—	"	0	0	0	0	3	24	37	6	1	3	7	0	0	0

TABLE III

Station 1287. Lat. 43°04′N., Long. 69°24′W.

		Sea	Sky	Sunrise	Sunset
Hauls A184–A218	July 10, 1932	Smooth	Clear	0431	1938
Hauls A219–A273	July 11, 1932	Moderate	Overcast, becoming clear	0432	1938
Hauls A274–A293	July 12, 1932	Slight	Clear	0433	1939

Number of copepods in thousands. *x* = less than 500.

Haul No.	Depth	Local Sun Time	Irrad. at Surface	Irrad. at Haul Depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus					Metridia lucens		
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Cop. III	Ad. ♂	Ad. ♀	Imm.
	m.		*μw*	*μw*	*per cent*											
A184	6	1418	15,800	7,400.	102	4	11	1	*x*	1	1	4	1	0	0	0
185	18	"	"	1,100.	"	3	4	0	0	1	1	4	4	0	0	0
186	30	"	"	170.	"	0	*x*	*x*	0	2	4	2	*x*	0	0	*x*
187	42	"	"	19.	"	0	0	0	*x*	1	3	2	0	9	50	12
188	54	"	"	—	"	0	0	0	*x*	1	5	1	0	13	141	25
189	66	1505	13,400	—	108	0	0	0	0	1	4	1	0	3	64	3
190	78	"	"	—	"	*x*	*x*	0	0	*x*	4	2	0	3	10	3
191	(Lost)															
192	102	"	"	—	"	0	0	0	0	0	6	5	*x*	0	1	2
193	114	"	"	—	"	*x*	*x*	*x*	0	0	11	5	0	*x*	2	1
199	6	1821	2,050	960.	147	4	7	*x*	0	1	1	2	2	0	0	*x*
200	18	"	"	140.	"	*x*	0	0	0	2	9	5	3	0	0	2
201	30	"	"	22.	"	0	0	0	0	*x*	4	1	*x*	0	1	1
202	42	"	"	2.5	"	*x*	0	0	*x*	1	2	2	*x*	8	45	21
203	54	"	"	—	"	0	0	0	*x*	1	2	1	0	24	89	18
204	6	1854	1,190	560.	173	2	6	0	0	*x*	1	3	1	0	*x*	0
205	18	"	"	80.	"	0	0	0	0	2	6	5	2	0	0	1
206	30	"	"	13.	"	0	0	0	0	1	9	3	0	0	4	2
207	42	"	"	1.4	"	0	0	0	0	0	3	2	0	10	78	22
208	54	"	"	—	"	0	0	0	0	1	3	2	*x*	6	48	13

TABLE III—*Continued*

Haul No.	Depth	Local Sun Time	Irrad. at Surface	Irrad. at Haul Depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus					Metridia lucens		
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Cop. III	Ad. ♂	Ad. ♀	Imm.
	m.		*μw*	*μw*	*per cent*											
209	6	1952	16	8.	267	5	7	2	0	1	1	11	5	0	*x*	1
210	18	"	"	1.1	"	0	*x*	*x*	*x*	3	2	1	1	0	3	1
211	30	"	"	—	"	0	0	0	0	1	12	2	*x*	0	34	3
212	42	"	"	—	"	0	0	0	0	1	8	3	0	3	91	12
213	54	"	"	—	"	*x*	*x*	0	0	1	2	1	0	13	23	14
214	6	2107	0	0	No light	4	7	1	0	1	1	6	3	0	6	2
215	18	"	"	0	" "	0	0	0	0	7	11	12	4	*x*	46	7
216	30	"	"	0	" "	0	0	0	0	5	21	5	0	0	48	5
217	42	"	"	0	" "	0	0	0	*x*	1	2	1	0	7	12	6
218	54	"	"	0	" "	0	*x*	0	*x*	*x*	4	3	0	12	12	9
219	6	0356	0	0	No light	6	5	1	*x*	2	1	4	1	0	3	1
220	18	"	"	0	" "	0	*x*	0	0	1	5	3	0	*x*	25	6
221	30	"	"	0	" "	0	0	0	0	0	2	1	0	*x*	9	3
222	42	"	"	0	" "	0	0	0	0	1	2	1	0	1	6	4
223	54	"	"	0	" "	*x*	0	0	*x*	1	1	1	0	1	4	3
224	6	0441	114	33.	No light	3	3	2	0	1	1	5	1	0	0	1
225	18	"	"	7.1	" "	*x*	1	0	*x*	2	3	3	*x*	*x*	7	1
226	30	"	"	1.0	" "	0	0	0	0	2	15	3	0	0	32	10
227	42	"	"	—	" "	0	0	0	0	3	8	2	0	2	42	14
228	54	"	"	—	" "	0	0	0	*x*	2	10	2	*x*	6	57	14
229	6	0540	800	230.	24	0	2	1	*x*	1	1	4	1	*x*	1	1
230	18	"	"	50.	"	0	*x*	0	*x*	2	1	2	1	0	7	1
231	30	"	"	7.4	"	0	0	0	0	1	5	3	0	0	12	3
232	42	"	"	1.4	"	0	0	0	0	1	4	1	0	1	17	7
233	54	"	"	—	"	0	*x*	*x*	*x*	1	2	1	0	3	9	5

TABLE III—*Continued*

Haul No.	Depth	Local Sun Time	Irrad. at Surface	Irrad. at Haul Depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus					Metridia lucens		
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Cop. III	Ad. ♂	Ad. ♀	Imm.
	m.		*μw*	*μw*	*per cent*											
234	6	0742	5,360	1,600.	49	2	2	*x*	*x*	*x*	1	4	2	0	0	0
235	18	"	"	330.	"	0	*x*	0	*x*	4	6	5	1	0	1	2
236	30	"	"	49.	"	0	0	0	0	5	12	5	0	*x*	39	7
237	42	"	"	9.1	"	*x*	0	0	0	3	5	2	0	2	51	7
238	54	"	"	—	"	0	0	0	0	2	5	2	0	2	68	10
239	6	1109	17,100	6,000.	Variable	1	1	0	0	0	*x*	3	2	0	*x*	*x*
240	18	"	"	1,800.	"	1	1	0	*x*	1	1	2	*x*	0	*x*	*x*
241	30	"	"	190.	"	*x*	0	0	*x*	7	33	9	0	0	1	5
242	42	"	"	38.	"	0	*x*	0	*x*	3	7	3	*x*	8	8	12
243	54	"	"	16.	"	0	0	0	*x*	2	5	3	0	6	40	6
244	6	1439	13,900	4,900.	95	1	1	*x*	*x*	*x*	1	7	2	0	0	0
245	18	"	"	1,500.	"	*x*	1	0	0	1	2	6	2	0	*x*	*x*
246	30	"	"	150.	"	0	0	0	0	4	9	3	0	0	3	9
247	42	"	"	31.	"	*x*	*x*	0	0	1	3	2	*x*	6	15	6
248	54	"	"	13.	"	0	0	0	*x*	1	3	3	0	15	55	8
249	66	1515	12,500	0.56	109	0	0	0	*x*	*x*	3	3	0	5	37	4
250	78	"	"	—	"	*x*	0	0	0	*x*	3	4	0	3	15	2
251	90	"	"	—	"	0	*x*	0	*x*	*x*	5	5	*x*	5	19	2
252	102	"	"	—	"	*x*	0	0	0	*x*	6	3	*x*	7	7	2
253	114	"	"	—	"	0	*x*	0	*x*	1	28	16	0	5	3	1
254	6	1821	1,820	660.	180	3	4	*x*	0	*x*	1	5	1	0	0	0
255	18	"	"	130.	"	0	1	0	*x*	3	9	6	1	*x*	0	1
256	30	"	"	9.8	"	0	0	0	0	7	18	2	0	0	98	12
257	42	"	"	2.9	"	0	0	0	*x*	2	9	2	0	*x*	106	18
258	54	"	"	—	"	0	0	0	*x*	*x*	7	*x*	0	7	134	16
259	6	1919	340	120.	300	3	6	1	*x*	1	1	5	2	0	0	0
260	18	"	"	24.	"	*x*	2	0	0	2	2	3	1	0	1	*x*
261	30	"	"	1.8	"	0	0	0	0	*x*	12	0	0	0	153	9
262	42	"	"	—	"	0	0	0	0	0	7	2	0	2	88	14
263	54	"	"	—	"	*x*	*x*	0	0	*x*	3	2	0	7	16	6

TABLE III—*Continued*

Haul No.	Depth	Local Sun Time	Irrad. at Surface	Irrad. at Haul Depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus					Metridia lucens		
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Cop. III	Ad. ♂	Ad. ♀	Imm.
	m.		μw	μw	per cent											
264	6	2022	0	0	(28 μ W at surface)	5	11	1	0	1	1	5	1	0	1	0
265	18	"	"	0		1	4	0	*x*	1	1	2	1	0	9	1
266	30	"	"	0		0	0	0	0	*x*	5	*x*	0	0	62	2
267	42	"	"	0		*x*	0	0	*x*	3	6	3	0	1	19	4
268	54	"	"	0		0	*x*	0	0	*x*	5	2	*x*	7	17	6
269	6	2126	0	0	No light	5	6	0	0	*x*	1	8	3	0	2	*x*
270	18	"	"	0	" "	1	3	*x*	*x*	1	*x*	2	1	0	2	*x*
271	30	"	"	0	" "	0	0	0	0	3	9	*x*	*x*	*x*	141	21
272	42	"	"	0	" "	0	0	0	1	1	3	2	0	2	28	9
273	54	"	"	0	" "	*x*	0	0	0	*x*	6	4	0	6	18	9
274	6	0337	0	0	No light	2	7	*x*	0	*x*	1	7	2	0	*x*	0
275	18	"	"	0	" "	*x*	*x*	0	0	1	1	3	*x*	0	60	3
276	30	"	"	0	" "	0	0	0	0	2	8	2	0	2	52	3
277	42	"	"	0	" "	0	0	0	0	1	7	3	0	4	21	5
278	54	"	"	0	" "	0	*x*	0	0	3	7	3	0	7	27	6
279	6	0428	124	33.	2	2	6	1	0	1	1	10	2	0	0	0
280	18	"	"	5.8	"	0	1	0	0	1	3	2	1	0	11	2
281	30	"	"	0.6	"	0	0	0	0	*x*	4	1	0	1	38	13
282	42	"	"	—	"	*x*	*x*	0	*x*	2	4	1	0	11	10	4
283	54	"	"	—	"	0	*x*	0	0	2	5	3	*x*	8	22	9
284	6	0542	2,360	640.	47	3	5	1	0	1	1	10	1	0	0	*x*
285	18	"	"	110.	"	*x*	1	0	0	1	2	1	*x*	0	*x*	1
286	30	"	"	12.	"	2	2	*x*	0	1	6	8	2	0	36	11
287	42	"	"	3.8	"	0	0	*x*	0	*x*	2	1	0	13	19	15
288	54	"	"	—	"	0	0	*x*	*x*	*x*	1	1	*x*	11	6	3
289	6	0739	8,930	2,400.	86	1	3	1	*x*	1	1	6	1	0	*x*	0
290	18	"	"	420.	"	*x*	*x*	*x*	0	1	1	1	1	0	0	1
291	30	"	"	46.	"	0	0	0	0	1	6	1	0	0	1	1
292	42	"	"	14.	"	0	0	0	*x*	*x*	2	2	0	0	105	32
293	54	"	"	2.4	"	0	0	0	*x*	0	2	2	0	3	29	7

From Fig. 3 it will be seen that at Station 1287 the adult females of *Metridia lucens* constitute the most numerous group (diagram plotted on half scale) of all the copepods. This group is distributed from 18 meters down to 114 meters, the greatest numbers occurring at the 54-meter level. The depth of maximum abundance of the adult males of this species is also 54 meters, but the range does not extend upwards so far and the animals are relatively more numerous at the lowest depths. All the immature *Metridia*[3] taken together show a maximum at 30 meters, thus exhibiting a tendency to inhabit the upper water layers more commonly than the adults. These observations on *Metridia* agree with Bigelow's conclusions (1924) that the level of plurimum abundance of this species as a whole in the Gulf of Maine is 50 to 150 meters.

Centropages typicus occurs in very much smaller numbers than either *Metridia* or *Calanus*. However, the tendency to concentrate toward the surface is very marked. This is in agreement with previous observations and is undoubtedly correlated with the warmer water found at the surface (Bigelow, 1924, and see below). The maximum numbers were taken at the 6-meter level, for both males and females, although the females were almost equally numerous at a depth of 18 meters. Immature individuals were present at the 6-meter level only.

Calanus finmarchicus is represented chiefly by immature forms, particularly copepodid stage V. The adult males occur in very small numbers at irregular intervals but increasing toward the lower limit of the range investigated (cf. Russell, 1928). The adult females show a maximum at 30 meters. The copepodid stages V and IV are found throughout the vertical range with marked maxima at 114 meters, but individuals of stage IV are relatively more numerous in the surface layers. Copepodid stage III is decidedly concentrated toward the surface. Great irregularities in the vertical distribution of *Calanus* in the Gulf of Maine have been reported previously. The present observations are consistent with Bigelow's conclusion (1924) that this species is far less plentiful in the surface stratum than below 10 meters, at this time of year.

From observations made on the penetration of light into the water at this station (Clarke, 1933), the depths at which intensities of 1000 microwatts, 100 microwatts, etc., occurred have been calculated and these are indicated by horizontal lines at the corresponding depths in

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[3] The numbers of immature individuals in the cases of *Metridia* and of *Centropages* were ordinarily too small to warrant division into age and sex groups. However, this was done for Stations 1285 and 1286 in the case of *Metridia*. I am indebted to Miss Mildred Campbell for working out this further analysis. The immature *Calanus* were divided into age groups for all hauls, following the stages described by Lebour (1916).

Fig. 3 (cf. Russell, 1933).[4] The relationship between the distribution of the copepods and the intensity of irradiation may thus be readily studied. It is seen, for example, that the zone of maximum abundance for adult *Metridia* exists in a region where the intensity of irradiation is between 1 and 10 microwatts. It must be borne in mind, however, that only blue light was measured. If the irradiation over the whole spectrum had been measured, the values obtained would have been greater and the change in light intensity with depth would have been found relatively more rapid, since blue light penetrates the farthest. Moreover, we do not know how this region of the spectrum compares with other regions in stimulating efficiency for copepods. The sensitivity of crustacea in general has been found to extend over a broad range but with a maximum usually in the yellow-green (cf. Bertholf, 1932; Lumer, 1932).

Measurements were made of the lengths of 25 or more individual copepods from each of the groups which were sufficiently numerous. Russell (1928) has shown that measurements of this sort may serve to identify seasonal broods. This information would therefore be useful

TABLE IV

Cephalothorax length of Metridia lucens adult female

Length	Night Hauls			Day Hauls				
	Haul 219 6 m.	Haul 220 18 m.	Haul 223 54 m.	Haul 246 30 m.	Haul 247 42 m.	Haul 248 54 m.	Haul 250 78 m.	Haul 253 114 m.
mm.								
1.743	—	—	—	—	1	—	—	—
1.785	—	—	—	2	2	—	—	—
1.828	—	—	1	—	2	1	—	2
1.870	3	—	—	7	3	5	1	3
1.913	3	2	4	12	13	16	—	5
1.955	2	5	2	10	8	21	3	8
1.998	7	4	4	7	12	24	4	6
2.040	6	5	7	6	4	14	10	3
2.083	1	4	1	2	1	8	7	6
2.125	2	2	6	2	3	3	5	5
2.168	—	2	—	—	—	2	6	5
2.210	1	1	—	1	1	4	6	2
2.253	—	—	—	1	—	2	6	4
2.295	—	—	—	—	—	—	2	—
2.338	—	—	—	—	—	—	—	1
No. measured	25	25	25	50	50	100	50	50
Av. length	2.001	2.037	2.020	1.965	1.958	2.001	2.114	2.046

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[4] For comparing light intensities 1 microwatt may be taken as approximately equal to 3 meter-candles as given by Russell, since his calculations have been based on the data of Poole and Atkins (cf. Clarke, 1933).

for comparison with subsequent investigations. Furthermore, it was desired to inquire whether size varied with depth within each group (cf. Gardiner, 1933). The frequency distributions of the lengths of the copepods are given in Tables IV, V, and VI together with the average lengths. The length of the cephalothorax (including lateral spine, if present) is the dimension considered and not the total length, since the former measurement can be made more quickly and accurately. Both the cephalothorax length and the total length (to the end of the caudal furcæ, but not including the furcal setæ) were measured for 100 individuals of adult female *Metridia lucens* and their ratio was found to be very nearly constant. The average cephalothorax length was 2.001 mm. and the average total length was 3.018 mm., giving an average ratio of 2 : 3 for this group.

From Table IV it may be seen that in the case of the adult females

Table V

Cephalothorax length of Metridia lucens and Centropages typicus

Length	Metridia lucens						Centropages typicus	
	Adult Male			Immature			Adult Male	Adult Female
	Haul 247 42 m.	Haul 250 78 m.	Haul 253 114 m.	Haul 247 42 m.	Haul 250 78 m.	Haul 253 114 m.	Haul 244 6 m.	Haul 244 6 m.
mm.								
1.063	—	—	—	—	1	—	—	—
1.105	—	—	—	—	—	—	—	—
1.148	1	—	—	—	—	—	—	—
1.190	—	—	—	—	—	—	—	—
1.233	1	—	—	1	—	—	5	—
1.275	8	4	4	—	—	—	4	3
1.318	14	14	6	2	1	—	5	1
1.360	1	3	7	4	2	2	8	—
1.403	—	—	5	2	3	—	2	3
1.455	—	1	3	5	3	—	1	8
1.488	—	3	—	5	10	5	—	3
1.530	—	—	—	3	4	9	—	6
1.573	—	—	—	1	—	3	—	1
1.615	—	—	—	—	—	2	—	—
1.658	—	—	—	—	—	—	—	—
1.700	—	—	—	—	—	—	—	—
1.743	—	—	—	—	—	—	—	—
1.785	—	—	—	1	1	—	—	—
1.828	—	—	—	—	—	—	—	—
1.870	—	—	—	1	—	1	—	—
1.913	—	—	—	—	—	1	—	—
1.955	—	—	—	—	—	2	—	—
No. measured	25	25	25	25	25	25	25	25
Av. length	1.296	1.342	1.356	1.466	1.458	1.583	1.319	1.445

of *Metridia lucens* the individuals in the deeper layers tend to be longer than those nearer the surface although the differences are very small. In the upper layers the average size is slightly greater at night than during the day. If the difference be regarded as significant, it could be interpreted as due to the presence at night of large individuals which had migrated up from the deeper layers. In the case of the adult males and the immature animals of this species there is a similar increase in the number of large specimens at the deeper levels (see Table V). For *Calanus finmarchicus* the data (Table VI) reveal a more pronounced grading effect. Here the difference in the lengths of the age stages may be clearly seen. In addition it will be noted that within each group the frequency distribution definitely moves in the direction of greater length with increasing depth. This observation agrees with that made

TABLE VI

Cephalothorax length of Calanus finmarchicus

Length	Adult Female			Stage V			Stage IV			Stage III
	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.
mm.										
1.148	—	—	—	—	—	—	—	—	—	1
1.190	—	—	—	—	—	—	—	—	—	5
1.233	—	—	—	—	—	—	—	—	—	5
1.275	—	—	—	—	—	—	—	1	—	6
1.318	—	—	—	—	—	—	—	—	—	5
1.360	—	—	—	—	—	—	—	—	—	3
1.403	—	—	—	—	—	—	—	1	—	—
1.445	—	—	—	—	—	—	—	—	—	—
1.488	—	—	—	—	—	—	—	—	—	—
1.530	—	—	—	—	—	—	1	1	—	—
1.573	—	—	—	—	—	—	5	—	1	—
1.615	—	—	—	—	—	—	7	4	--	—
1.658	—	—	—	—	—	—	5	1	1	—
1.700	—	—	—	—	—	—	4	2	5	—
1.743	—	—	—	—	—	—	1	6	3	—
1.785	—	—	—	—	—	—	2	3	2	—
1.828	—	—	—	—	—	—	—	2	6	—
1.870	—	—	—	—	—	—	—	4	3	—
1.913	—	—	—	—	—	—	—	—	2	—
1.955	—	—	—	4	—	1	—	—	1	—
1.998	—	—	—	3	—	—	—	—	—	—
2.040	—	—	—	2	2	1	—	—	—	—
2.083	—	—	—	2	1	—	—	—	—	—
2.125	1	—	—	2	1	—	—	—	—	—
2.168	2	—	—	1	2	1	—	—	—	—
2.210	1	—	1	3	4	3	—	—	—	—
2.253	2	—	—	3	1	1	—	—	—	—

TABLE VI—*Continued*

Length	Adult Female			Stage V			Stage IV			Stage III
	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.
mm.										
2.295	3	—	—	1	2	4	—	—	—	—
2.338	—	—	—	—	2	5	—	—	—	—
2.380	2	4	—	1	1	1	—	—	—	—
2.423	2	2	1	3	4	3	—	—	—	—
2.465	—	3	2	—	2	3	—	—	—	—
2.508	2	—	1	—	1	—	—	—	—	—
2.550	2	1	—	—	1	—	—	—	—	—
2.593	1	3	1	—	1	1	—	—	—	—
2.635	4	2	1	—	—	1	—	—	—	—
2.678	1	4	2	—	—	—	—	—	—	—
2.720	1	1	—	—	—	—	—	—	—	—
2.763	—	3	2	—	—	—	—	—	—	—
2.805	1	1	5	—	—	—	—	—	—	—
2.848	—	1	1	—	—	—	—	—	—	—
2.890	—	—	3	—	—	—	—	—	—	—
2.933	—	—	—	—	—	—	—	—	—	—
2.975	—	—	1	—	—	—	—	—	—	—
3.018	—	—	2	—	—	—	—	—	—	—
3.060	—	—	—	—	—	—	—	—	—	—
3.103	—	—	—	—	—	—	—	—	—	—
3.145	—	—	—	—	—	—	—	—	—	—
3.188	—	—	1	—	—	—	—	—	—	—
3.230	—	—	1	—	—	—	—	—	—	—
No. measured	25	25	25	25	25	25	25	25	24	25
Av. length	2.445	2.589	2.766	2.152	2.305	2.328	1.644	1.707	1.787	1.263

by Gardiner (1933) for stage V of this species and indicates that the tendency is of general application for the other stages.

Data on the vertical variations of the following physical and chemical factors were secured by Dr. A. C. Redfield: temperature, salinity, pH, oxygen, nitrate, nitrite, and PO_4. This information is presented graphically in Fig. 4. The temperature—which changes so abruptly between 10 and 20 meters—is probably the most important of these as an agent in determining the vertical distribution of the plankton organisms under consideration (cf. Bigelow, 1924).

It was not possible to obtain measurements of the abundance of the phytoplankton at this station, but observations were made in this vicinity about three weeks later by Gran (1933). He found that the phytoplankton maximum occurred at 20 to 30 meters. The dominating species were *Ceratium fusus*, *Pontosphæra Huxleyi*, and germinating cystæ of *Gonyaulax orientalis*.

Diurnal Migration

In Fig. 5 the changes in the vertical distribution of the adult females of *Metridia* during the 48-hour period comprising Station 1287 are represented diagrammatically. Since this is the most numerous group of copepods, more reliance can be placed upon these observations than upon those of other groups. The first series of hauls made early in the afternoon of July 10 showed that these animals were inhabiting the water from 30 meters down to 54 meters, with maximum abundance at this lower depth. As the afternoon progressed and night came on, their range was extended upwards to the 6-meter level. At the same time the zone of maximum abundance rose gradually. At 21 o'clock the greatest numbers were taken at 30 meters and at 4 o'clock the next morning the maximum occurred at 18 meters. The

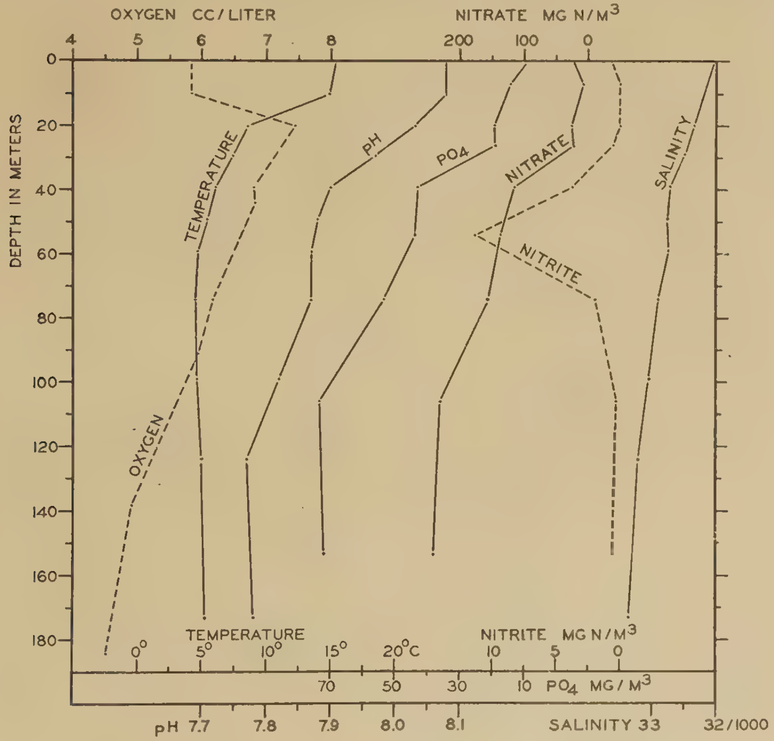

FIG. 4. Variation of physical factors with depth.
Station 1287 (except oxygen which is for Station 1285).

The pH measurements were made by a spectrophotometric method using cresol red as an indicator. The pK value of cresol red was taken as 8.13 and corrected for salinity on the assumption of a salt error of − 0.27. The solutions were measured at a temperature of 20° C. The values are not corrected for the change in temperature.

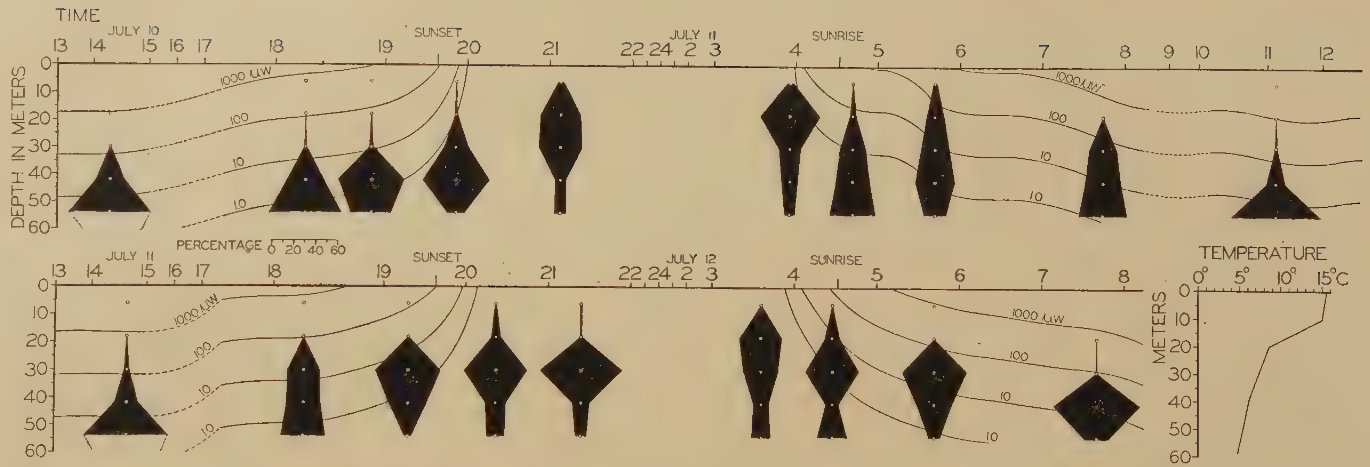

Fig. 5. The diurnal migration of the adult females of *Metridia lucens* during the 48-hour period of Station 1287 (July 10, 11, and 12, 1932). The position of the maximum early each afternoon is shown to be 54 meters, and not lower, by the deep series made immediately afterwards (see Table III). The changes in the light intensity are indicated by the lines representing the depth at which 1000 microwatts, 100 microwatts, etc., occurred. (One microwatt may be taken as approximately equal to three meter-candles as used by Poole and Atkins, 1929.) Where the lines are broken, a shortening of the time scale is indicated merely; observations were not discontinued during these periods. The temperature curve for this station is reproduced in the lower right-hand corner.

next series of hauls revealed the beginning of the downward migration. It is seen that the level of maximum abundance fell and the upper layers of water were deserted progressively toward noon. The vertical distribution of female *Metridia* at 0441, with a maximum indicated at 54 meters, does not conform to the gradual change of abundance which is indicated by the other series. The explanation for this is wanting; no corresponding irregularity occurred on the following morning. Early in the afternoon of July 11 the distribution of the animals was found to be very nearly the same as on the previous day. Toward night the animals migrated upward in similar fashion although there are certain differences in the configuration of the population diagrams. As before, the zone of maximum abundance reached the highest level (18 meters) just before dawn. The downward migration ensued as on the previous day, although here again certain differences in distribution do appear.

From the observations of the irradiation on deck and the transparency of the water (tested five times during this station), lines of equal light intensity have been drawn in Fig. 5. An idea can thus be obtained of the actual changes in irradiation to which the organisms were subjected during the course of each day. Inspection of the diagram reveals the fact that in general the zone of maximum abundance of *Metridia* occurs between light intensities of 10 microwatts and 1 microwatt (or less) and rises and falls coincidently throughout the day (cf. Russell, 1927).

At 20 o'clock on July 10 the greatest number of copepods was found below this range of light intensity and at 6 and 8 o'clock on July 12 the maximum was above it. This might be taken to indicate that the animals could not swim fast enough to retain their position within the usual limits of irradiation at those times of day when the light is changing most rapidly. For example, it can be roughly calculated from the figure that the position of the 1-microwatt line rises about 46 meters in the hour between 19 and 20 o'clock on July 10. To keep pace with this a copepod would have to swim vertically upwards at an average speed of 77 cm./min. Parker (1902) found in the laboratory that *Labidocera* swam upwards at the rate of 10 cm./min. The speeds of ascent of the Lucerne plankton observed by Worthington (1931) are even slower than this. However, Welsh (1933) has found that *Centropages typicus* swims toward a light for a distance of 10 cm. in a horizontal trough at average speeds of 71 cm. to 136 cm./min., depending upon the intensity of the light. Such an animal would theoretically be able to swim upwards at a speed equal to this rate minus the rate of sinking due to gravity. Parker observed etherized *Labidocera* to sink at 30 cm./min. and Gardiner (1933) has shown that large specimens of *Calanus* in an anæsthetized condition sink at 50 cm./min. If the sinking rate of *Centropages* is no faster than this, it would appear that this species could ascend at a speed as great as that at which the light changes in the water. Downward swimming at such a rate could probably be easily accomplished. It should be noted that on July 11 no tendency for the zone of maximum abundance of *Metridia* to lag behind the usual range of irradiation is indicated either in the morning or in the evening.

The total range for the population of female *Metridia* never extends above the 1000-microwatt line and it often falls short of this. This value may therefore represent the greatest intensity of light which these organisms can withstand at this period of the year. The copepods do not appear to have been restricted to regions of lower light intensities in the early morning than they were later in the day (cf. Worthington, 1931), thus furnishing no evidence that the organisms became less sensitive to light as the day progressed (cf. Russell, 1931).

The observations consistently indicate that the animals did not tend to become uniformly distributed after complete darkness (cf. Russell, 1926) but continued to migrate toward the surface, reaching their highest average level just before dawn. However, the zone of maximum abundance never rose above 18 meters and very few individuals were taken at the 6-meter level. The stratum of warm water (15° C.) present at the surface, sharply delineated between 10 and 20 meters from the cold water (5° to 8° C.) beneath it (cf. Fig. 5), undoubtedly played a large part in deterring the animals from moving much above the 18-meter level (cf. Russell, 1927; Bigelow, 1924).[5] The downward migration in the morning did not begin before the irradiation in the surface layers had become measurably intense, but soon after this a rapid descent was initiated, for a considerable movement had been accomplished by sunrise.

The behavior of the other groups of copepods is less clearly defined. At Station 1053 (see Table I) *Centropages* was caught in very large numbers in the surface layers. Just before sunrise the great majority of animals in each group was found at a depth of 4.5 meters. As the day progressed, a gradual downward migration took place until noon when the zone of maximum abundance occurred at its greatest depth of 23 meters. In each case a number of animals constituting a secondary maximum remained near the surface. The downward movement of *Calanus* during this period was of much smaller proportions. Furthermore, such migration as did occur began later than in the case of *Centropages*. This may represent the difference in time before effective intensities of light reached the deeper levels where the majority of *Calanus* were living. The hydrographic data for this station are as follows:

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[5] Mr. Russell suggests an alternative interpretation of these observations. He concludes from the diagrams that by dawn the distribution in the deeper layers is tending to uniformity and that if there had been no thermocline the extension of a certain proportion upward would have produced a uniform distribution. The objection to this view is that the animals present at the 30-meter level at 21 o'clock did not spread upwards and downwards in equal numbers during the night but the great majority of them moved up to the 18-meter level.

Depth *meters*	Temp. ° *C.*	Salinity *mille.*
0	16.81	31.97
9	16.82	31.92
17	16.80	31.96
35	16.30	31.96
52	10.60	32.24
69	—	32.31
86	5.86	32.50
104	5.17	32.68

It is evident that the downward migration of *Centropages* was not limited by these factors and that the range of *Calanus* extended right across the thermocline. Very few specimens of *Metridia* were taken at this station.

During the 24-hour period comprising Stations 1285 and 1286 (see Table II) *Centropages* exhibited no vertical migration whatever, maximum numbers being taken in the uppermost net at the four times that observations were made. On the other hand, *Calanus* was taken in every haul at this station. There were extremely few adult males, and the small numbers of adult females taken were distributed irregularly, apparently undergoing no definite migration during the period. However, the groups composed of copepodid stages V and IV respectively showed a fairly regular distribution with a definite maximum in each case. The largest numbers occurred at 95 meters at noon, at 85 meters at dusk, at 65 and 75 meters toward midnight, and at 65 meters the following morning. These forms therefore inhabited slightly greater depths at noon than at other times. The data for the various groups into which the *Metridia* have been divided permit only a similarly general conclusion to be drawn. The zones of maximum abundance for adult males and for adult females existed at 96 meters at noon and at various shallower depths at other periods of the day. The distribution of the copepodid stages is seen to be very irregular.

During the 48-hour period of Station 1287 *Centropages* was found confined to the surface layers as before. In the series made on the first day the range of the adults of this species extended to 18 or 30 meters at noon while the population was chiefly limited to the 6-meter level at other times. This statement does not hold true for the second day, however. The immature group did not leave the uppermost level at any time.

In the case of the adult females of *Calanus* there appears to have been a fairly consistent movement of the zone of maximum abundance from 30 meters at noon, to 6 meters at four o'clock the next morning, and then down to 30 meters again the following noon. After this, however, the maximum occurs irregularly at a variety of depths. Copepodid stage V is represented in every series at all depths. In many cases the population is rather evenly distributed throughout the range. The maximum for each series occurs at the 30-meter level in the majority of cases without regard to time of day. Copepodid stage IV was taken in all but one haul at this station but distribution was irregular. Maxima were found at the 6-meter level for the most part. The prominent maximum found at 114 meters for both stages V and IV may constitute a population which remained at this low level throughout each day and which is distinct from the secondary maximum usually found near the surface on each afternoon. Possibly the animals comprising the latter are those which form the single maximum found regularly in the shallow series made at other periods of the day. The group composed of copepodid stage III was characteristically found in the surface strata. The copepodid stages therefore either failed entirely to migrate, or at most, they moved downwards a short distance during the middle of the day as at the previous station.

The vertical distribution of *Metridia* tends to be more consistent than in the case of *Calanus*. The adult males of *Metridia* are confined to a relatively narrow range. Apparently no diurnal migration was carried out by this group, however, for the maximum in each series except five at this station was found at 54 meters. The pronounced migration of the adult females has already been discussed in detail. The occurrence of the zone of maximum abundance for the immature of this species defies generalization; but it may be concluded that the upper limit of the vertical range of this group exists at a lower depth during the middle of the day than at night.

Discussion

The detailed study of the vertical distribution of these various sex- and age-groups of copepods impresses one with the remarkable differences in behavior exhibited. However, such lack of uniformity among different species, within the same species, and even between different broods, has frequently been reported (*e.g.* Russell, 1928, 1932;

Worthington, 1931; and Southern and Gardiner, 1932). As an example of the differences in behavior of the same species as found by different investigators, our observations on *Calanus finmarchicus* may be compared with those of Russell (1926, 1927). This worker found that the depth of maximum abundance of this species in the Plymouth area changed from about 15 meters at noon to the very surface at night. But according to the data presented above *Calanus* was ordinarily distributed irregularly throughout the upper 50 meters of water and showed only a slight tendency to migrate. Bigelow (1924) has reported considerable lack of conformity in day and night catches of *Calanus* in the Gulf of Maine. In general he finds that ". . . the reactions of *Calanus* in their local application to the gulf result in its being far less plentiful in the surface stratum than below 10 meters or so by day, and often by night, during the half of the year when the temperature is highest and the solar illumination brightest."

Although the nature of the diurnal migration of plankton organisms varies so profoundly, and although different explanations are evidently required for the details of each individual case, still the possibility exists that certain fundamental principles can be found which underlie the phenomenon in its broad outlines. Accordingly, the results of the present investigation will be briefly examined for evidence supporting any of the theories which have been advanced previously.

The absence of vertical movements in the case of some of the animals under circumstances when other forms are migrating is itself a proof that, whatever causal factors may be operating, they are not of uniform application nor effectiveness. Thus, although change of light intensity may have caused the migration undertaken by *Metridia*, it was not equally effective in producing a vertical movement of *Calanus*. Bigelow (1924) says of this species, ". . . its absence on the surface in the regions where it swarms in deeper water is not caused altogether by sunlight, for while it probably does tend to descend during the most brilliantly illuminated hours, on several occasions we have made rich catches on the surface when the sun was high in the sky."

Let us therefore confine our search for a general explanation to those organisms which do migrate, and furthermore to those which move consistently toward the surface at night and downward in the morning. Because of their large numbers and relatively consistent behavior the adult females of *Metridia* form the group from which deduction may most legitimately be drawn in the present investigation. The description of the migration of these animals already presented reveals the fact that the vertical movements definitely coincide with the periods of day and night. Hydrographic measurements made on the preceding days by Dr. A. C. Redfield[6] showed that no significant diurnal changes in the hydrogen-ion concentration, in the oxygen content, or in the temperature were taking place in this region. It seems justifiable to conclude, therefore, as the majority of previous investigators have done, that light is the primary physical factor controlling the vertical migration of these organisms.

The behavior of *Metridia* provides evidence that, in the present case at least, other factors besides light were operating. For, as has already been seen, these animals continue to move toward the surface even after complete darkness and are found at their highest level just before dawn. This type of behavior was found by Worthington (1931) and by Southern and Gardiner (1932) for certain freshwater forms, but is in sharp contrast with observations of Russell (1927) in the Plymouth area, in which many plankton animals were found uniformly distributed in the vertical column of water at midnight. Continued migration after dark may be due to a negative geotropism, as has been suggested by Worthington and others. The possibility of such behavior being correlated with feeding habits (cf. Worthington, 1931; and Southern and Gardiner, 1932) should be examined for *Metridia*. As stated above, the phytoplankton maximum in the present case occurred at a depth of 20 to 30 meters. The upper limit of the vertical movement of *Metridia* corresponds roughly to the upper limit of this zone, but it also corresponds approximately to the beginning of the thermocline (cf. Fig. 5). Pronounced changes in temperature undoubtedly are effective in limiting migrations (cf. Russell, 1927). Furthermore, we have very little information on the actual food of particular species of copepods. More information must be obtained before the importance of feeding reactions in this connection can be decided.

In so far as light is regarded as effective in controlling diurnal migrations, it is worth while to consider in what manner it may exert its influence. The theory that plankton animals tend to keep within a range of "optimum intensity" of light (Russell, 1926, 1927), although supported by a considerable mass of evidence, has failed to fit the facts in certain cases (cf. Worthington, 1931). Question has also been raised as to the acceptability of the "optimum intensity" theory on the basis of experimental work in the laboratory (Clarke, 1930).[7] In the present investigation, however, *Metridia* were found fairly consistently within a definite range of irradiation, as has already been pointed out.

[6] Unpublished data.

[7] Due to an error, line 26, page 128, of this report became transposed. It should read: ". . . due simply to a negative phototropism and a positive geotropism produced by the rising of the sun in the morning. . . ."

According to other theories, based in large part on laboratory experiments, light is supposed to exert its effect indirectly, by modifying the reactions of the animals to such factors as gravity and temperature (cf. Dice, 1914; Esterly, 1919; Rose, 1925). Whether the control by light is regarded as indirect or as direct, an important distinction should be made between two possible ways in which it may act, viz.: by virtue primarily (1) of its *change* of intensity, or (2) of its *absolute magnitude* of intensity (cf. Clarke, 1930, 1932). The behavior of plankton organisms may be regarded as a series of similar responses to changes in the intensity of light or as different responses to constant intensities of different magnitudes.

Since responses to change of irradiation are ordinarily of short duration, it would be necessary, in order for the first possibility to hold true, for the increase or decrease of light intensity to take place continuously at a rate above the threshold. The requisite rate of change for *Daphnia magna* may be calculated from existing data (Clarke, 1930) and is found never to be less than 16 per cent per minute. In the sea the irradiation changes at a rate which varies according to the time of day but which is ordinarily greatest at sunrise and sunset (cf. Fig. 5). In order to ascertain whether the change of light intensity in the water ever proceeds at a rate above this threshold, a calculation may be made for a time when irradiation is still measurably intense but changing at a maximum speed for that intensity. Between 1900 and 1940 o'clock on July 10 at a depth of 30 meters the irradiation was reduced from 10 microwatts to 1 microwatt. This represents an average rate of change of 4 per cent per minute. If the responses of *Metridia* are to any degree commensurate with those of *Daphnia*, it would appear that the rate of change of light in the sea at no time rose above the threshold value. Nevertheless, an upward movement of the plankton took place during this time. Ewald (1910), recognizing these difficulties, gives reasons for believing that the sensitivity of the animals is increased in nature and that even a gradual change in light intensity might evoke the responses. Judgment on this question must be reserved until more information is available.

On the other hand, if the *change* of irradiation is rejected as a source of stimulation, then the problem resolves into one of the effect of different sensibly constant intensities upon the phototropism, geotropism, etc., of the organisms. In cases, such as *Daphnia*, in which the signs of the tropisms are unchanging under all intensities of light, and in which the animal is able to adapt to light over a wide range, an appeal to these reactions can not account for vertical migration, whether the fundamental feature of it be regarded as a tendency to maintain a position within a certain zone of light intensity or as some other type of behavior. Although these experiments have not yet been proved to give a true picture of the behavior of plankton animals in nature, carefully controlled laboratory investigations of this type have already shed much light upon various aspects of the problem. Whatever theory may be advanced to explain diurnal migration, the underlying reactions involved must be demonstrated conclusively in the laboratory before the explanation can be finally accepted.

SUMMARY

1. Observations on the vertical distribution of copepods and measurements of the submarine irradiation were made in the Gulf of Maine during 12- to 48-hour periods to procure information on the importance of light in controlling diurnal migration.

2. Five closing nets were towed simultaneously at different depths from a single vertical cable. The method devised for allowing the nets to be open only during the 10-minute towing period is described.

3. The sources of error involved in the work at sea and in the sampling of the catch in the laboratory are discussed. The methods used allow special dependence to be placed upon differences found among the hauls within each series.

4. The general vertical distribution of the three species studied was as follows: *Centropages typicus* inhabited the stratum of water above the thermocline (10 to 20 meters), *Calanus finmarchicus* was irregularly distributed, and *Metridia lucens* occurred below the thermocline. In each case the individuals present in the upper part of the range were found to have a slightly smaller average length than those occurring at deeper levels.

5. The adult females of *Metridia* exhibited the most marked diurnal migration, the level of maximum abundance rising in the afternoon and during the night and falling in the morning. These movements were found to coincide to a considerable extent with changes in submarine irradiation.

6. The changes in the vertical distribution of the other groups of copepods were slight or quite irregular. In some cases, however, there was a definite tendency for the maximum to occur at greater depths at noon than at other times.

7. This investigation confirms the idea that light is the most important factor controlling diurnal migration. In addition, the observations are shown to have a bearing on various of the theories regarding the manner in which light exerts its effect.

I am indebted to the Woods Hole Oceanographic Institution for the purchase of the closing nets and for the opportunity to make the observations from the "Atlantis" and to the Laboratory of General Physiology, Harvard University, for facilities for the preparation of the report.

I am extremely grateful to Dr. E. J. Allen, Mr. F. S. Russell, and other members of the Marine Biological Association for the use of the facilities of the Laboratory at Plymouth and for a great deal of assistance in assembling equipment when the "Atlantis" was preparing for sea, and I wish to thank Mr. Russell in addition for valuable advice in planning the methods and program of work and for most helpful criticism of the manuscript.

I am indebted to Dr. A. C. Redfield of the Woods Hole Oceanographic Institution for furnishing me with the hydrographic data and for criticizing the manuscript, and to Mr. C. O'D. Iselin, not only for assistance in designing the closing nets but also for a great deal of help in all the operations at sea. Miss Mildred Campbell of the University of Toronto very kindly analyzed the entire catch made at Stations 1285 and 1286 for me. Mr. C. L. Wheeler and Mr. D. Lillie made the measurements of the copepod lengths. In addition, I wish to thank Mrs. G. L. Clarke, Mr. D. B. Merriman, Mr. F. Bigelow, and Mr. C. L. Wheeler for much assistance in the collection and analysis of the catch and in the preparation of this report.

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Contribution No. 37

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THE COLOR CHANGES OF ELASMOBRANCH FISHES

BY G. H. PARKER

WOODS HOLE OCEANOGRAPHIC INSTITUTION[1]

Communicated October 28, 1933

Very little work has been done on the color changes of elasmobranch fishes. In 1921 Schaefer reported in an appendix to an extended article on the color changes in flatfishes that the skates Raja clavata and Raja batis showed on being tested no such changes. A little over a decade later Lundstrom and Bard (1932) described striking color changes in the dogfish Mustelus canis and showed that in this fish the secretions from the pituitary gland were accountable for the dark phase of this fish. The light phase of Mustelus was studied in 1933 by Parker and Porter who in a paper now in press (1934) have demonstrated that this coloration is induced by the direct action of nerves. In the course of the work on dogfishes opportunity was found to test the possibility of color changes in the common skate Raja erinacea which was available in the laboratory at that time.

Two individuals, indistinguishably dark brown, were placed one in a white-walled sea-water tank illuminated from above and the other in a similar black-walled tank. After eighteen hours the skate in the white tank was light brown and that in the black tank was dark brown. The fishes were then transferred each to the other tank. After the light skate had been in the dark tank nine hours it became decidedly dark, but the dark skate after an equal interval in the light tank remained dark. Twelve hours later, however, this fish had become very light and of a hue that could be described as pinkish white. At this stage in the tests unfortunately the mate of this light fish died.

The light fish, now extremely pale in tint, was transferred to the black tank. In two hours it had darkened considerably and in twelve hours it was again dark brown. In consequence of the need of the tanks for other experimental work, tests on this skate had to be discontinued. Enough, however, had been observed on these two fishes to justify the conclusion that Raja erinacea, like Mustelus canis, is subject to reasonably striking color changes. From this standpoint it would seem desirable to retest the European forms previously reported on by Schaefer to ascertain whether upon close examination they, too, might not show color changes.

[1] Contribution No. 37.

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Report for the years 1930-1932

THE
WOODS HOLE OCEANOGRAPHIC INSTITUTION

REPORT FOR THE YEARS
1930-1932

TABLE OF CONTENTS

PAGE

I Trustees, as of December 31, 1932 3

II Act of Incorporation 4

III By-Laws 5

IV Report of the Treasurer 10

V Reports of the Director 14

VI Staff, as of December 31, 1932 29

VII Members of the Corporation, as of December 31, 1932 . . . 30

Appendix A: Reports of progress by investigators working at the Institution during 1931 31

Appendix B: Reports of progress by investigators working at the Institution during 1932 38

I

TRUSTEES

(As of December 31, 1932)

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Lawrason Riggs, Jr., Treasurer, 25 Broad Street, New York, N.Y.
Henry B. Bigelow, Clerk of the Corporation, Woods Hole, Mass.

II

Act of Incorporation

THE COMMONWEALTH OF MASSACHUSETTS

BE IT KNOWN That whereas

Ralph G. Boyd, Elizabeth D. Peabody,
Charles F. Dunbar, William E. Hays,
E. Curtiss Mower, Jr., Bertha A. Patten
and E. Louise Malloch

have associated themselves with the intention of forming a corporation under the name of the

WOODS HOLE OCEANOGRAPHIC INSTITUTION,

for the purpose of the following:—To prosecute the study of oceanography in all its branches; to maintain a laboratory or laboratories, together with boats and equipment and a school for instruction in oceanography and allied subjects; to accept and hold money, and property of any kind whatsoever, and wherever situated, and whether received through bequest, devise, gift or otherwise; and to apply from time to time and at any time to the purposes of the corporation, or to any of them, all or any part of the income and/or principal of any funds and/or property held by the corporation; and have complied with the provisions of the Statutes of this Commonwealth in such case made and provided, as appears from the certificate of the

Proper Officers

of said corporation, duly approved by the Commissioner of Corporations and Taxation and recorded in this office:

NOW, THEREFORE, I, Frederick W. Cook, Secretary of the Commonwealth of Massachusetts, DO HEREBY CERTIFY that said

Ralph G. Boyd, Elizabeth D. Peabody,
Charles F. Dunbar, William E. Hays,
E. Curtiss Mower, Jr., Bertha A. Patten
and E. Louise Malloch,

their associates and successors, are legally organized and established, and are hereby made, an existing corporation under the name of the

WOODS HOLE OCEANOGRAPHIC INSTITUTION,

with the powers, rights and privileges, and subject to the limitations, duties and restrictions, which by law appertain thereto.

WITNESS my official signature hereunto subscribed, and the Great Seal of The Commonwealth of Massachusetts hereunto affixed, this sixth day of January in the year of our Lord one thousand nine hundred and thirty.

(signed) F. W. COOK

(L.S.) Secretary of the Commonwealth

III

BY-LAWS

Article I

OFFICERS AND THEIR ELECTION

Section 1. Officers. The officers of the corporation shall be a Board of Trustees, a Director, a Treasurer and a Clerk (none of whom need be incorporators or members of the corporation), a President, who shall be chosen always from the Board of Trustees, and such other officers as the Board of Trustees may from time to time appoint. Any two or more of the offices of Director, Treasurer, Clerk and President may be held by the same person. The Director, Treasurer and Clerk shall be ex officio members of the Board of Trustees additional (except when Trustees by election also) to the members of such Board elected pursuant to the provisions of the following paragraph.

Section 2. Elections. The incorporators shall elect three Trustees and these three, together with such additional persons, if any, as at the time of such election are already Trustees ex officio, shall constitute the first Board of Trustees. The Board of Trustees may from time to time prior to the first annual meeting of members of the corporation, to be held in 1930, elect additional Trustees to a number not exceeding in the aggregate twenty-one (21), and, subject to the limitation aforesaid, the Trustees present at any meeting of the Board, if constituting a quorum of the Board as it existed at the beginning of the meeting, may elect any number of additional Trustees, notwithstanding that none of the persons elected at the meeting were present to participate in any of its proceedings. At said first annual meeting twenty-four (24) Trustees shall be chosen, who shall be divided into four classes, each consisting of six (6) persons, to serve one, two, three and four years respectively, and thereafter at each annual meeting of members of the corporation, six (6) Trustees shall be elected to hold office for the term of four (4) years. The Trustees chosen pursuant to the provisions of this paragraph prior to said first annual meeting shall hold office until said annual meeting and until a new Board shall be elected as in this paragraph provided. The Trustees chosen at said annual meeting, or at any subsequent meeting of members of the corporation shall hold office until the end of the period for which they are elected and until their successors are chosen.

The first Treasurer and first Clerk shall be elected by the incorporators and shall hold their respective offices until said first annual meeting and until their respective successors are elected and qualified. At each annual meeting of the members of the corporation said members shall elect a Treasurer and a Clerk, who shall hold their respective offices until the next annual meeting and until their respective successors are elected and qualified.

The President and the Director shall be from time to time elected by the Board of Trustees and each shall hold his office until the Trustees shall have voted to terminate his tenure thereof or shall have elected his successor.

The election of all officers herein designated by title shall be by ballot. Any officer may resign by presenting a written resignation to the President, Clerk, or Board of Trustees, such resignation to take effect at the time therein indicated.

Section 3. Vacancies. Every vacancy occurring in the Board of Trustees as at any time constituted prior to said first annual meeting may be filled by the Board of Trustees at any meeting and the person chosen to fill such vacancy shall hold office until said first annual meeting and until a new Board of Trustees shall have been elected.

Every vacancy occurring in the Board of Trustees after the election of the four classes of Trustees, as hereinbefore provided, may be filled by the Board of Trustees at any meeting and the person chosen to fill such vacancy shall hold office until the expiration of the term for which his predecessor was elected and until his successor has been elected.

Every vacancy occurring in the office of Treasurer or of Clerk may be filled by the Board of Trustees and the person chosen to fill such vacancy shall hold office until the next annual meeting of the members of the corporation and until his successor is chosen and qualified.

Article II

MEMBERS' MEETINGS

Section 1. Annual Meetings. The Annual Meeting of the members of the Corporation shall be held at the office of the corporation at Woods Hole, Massachusetts, or at such other place in Woods Hole as the Trustees may have designated as a place for holding such meetings, on the Thursday following the second Tuesday in August of each year, at twelve o'clock, noon.

Section 2. Special Meetings. Special Meetings of the members of the corporation may be called by the Trustees, to be held respectively at such places in Woods Hole or Boston, Massachusetts, and at such times as the respective calls may designate.

Section 3. Quorum and Voting. At any meeting of the members of the corporation a majority of the then members of the corporation, if present in person or by proxy, shall constitute a quorum for the transaction of business. For the purposes of all elections and other matters left to the decision of the members of the corporation each member shall be entitled to one vote.

Section 4. Proxies. All proxies shall be in writing signed by the members giving them.

Section 5. Adjournments. Reasonable adjournments may be made from time to time of any meeting of the members of the corporation.

Section 6. Notices. Notice of the time and place of every meeting of members of the corporation shall be served on each member by the Clerk, or by some officer of the corporation authorized by the Trustees to serve such notices, by delivering to him personally or mailing, postage prepaid, addressed to him at his address as registered on the books of the corporation (or, in the case of a member who has no such recorded address, at his last known address) a written or printed notice, not less than ten (10) days before the day fixed for the meeting. Notices of special meetings of the members shall state the purposes thereof.

Article III

TRUSTEES' MEETINGS

Meetings of the Board of Trustees shall be held at such place within or without Massachusetts as may from time to time be fixed by resolution of the Board, or as may be specified in the call of any meeting. Meetings of the Board may be held at any time upon call of the President or any two (2) Trustees made by giving notice (or causing notice to be given by the Clerk or the Secretary, if any, or by any other person) to each Trustee (other than the person or persons making the call) of the time and place of the meeting, written or printed and mailed to his last known address, postage prepaid, at least three (3) days before the day of the meeting. Seven (7) Trustees (or, at any time when there are in office less than thirteen (13) Trustees, a majority of the Trustees then in office) shall constitute a quorum for the transaction of business. In all matters to be acted upon by the Board of Trustees, each Trustee, regardless of whether he holds that office by reason of election thereto, or by reason of holding one or more other offices, or for both said reasons, shall be entitled only to one vote.

Article IV

POWERS AND DUTIES OF OFFICERS

Section 1. President. The President when present shall preside at the meetings of the Board of Trustees and of the members of the corporation.

Section 2. Treasurer. The Treasurer, subject to the control of the Trustees, shall have custody of the money and books of account of the corporation and keep full, regular and accurate accounts of the financial transactions of the corporation and keep the funds of the corporation other than permanent funds invested in investments legal in one or more states for the investment of trust funds.

Section 3. Clerk. The Clerk, who shall be a resident of Massachusetts and be sworn to the faithful performance of his duties shall keep a true record of all meetings of the Board of Trustees and of the members of the corporation at which he is present. In his absence from any meeting a Clerk pro tempore shall be chosen and need not be sworn.

Section 4. Trustees. The Trustees shall have the control and management of the property and affairs of the corporation. Subject only to the foregoing provisions of these By-Laws they may choose such officers and agents as they think best, fix the compensation and define the powers and duties of all officers and agents and at any time remove them, or any of them, from office. They shall cause a report of the condition of the corporation to be presented at each annual meeting of the members of the corporation.

Article V

MEMBERS OF THE CORPORATION

The Board of Trustees may from time to time elect members to the corporation upon such terms and conditions as they think best. The Incorporators shall not be deemed members of the corporation and shall not be entitled to receive notice of or to vote at any of the meetings of members of the corporation provided for in *Article II* hereof.

Article VI

EXECUTIVE COMMITTEE

The Board of Trustees may at any time and from time to time designate an Executive Committee, to consist of three (3) or more of the Trustees, as the Board may from time to time determine. The Executive Committee shall have such of the powers of the Board of Trustees as the Board may from time to time delegate to it. Said Committee may make such rules as it sees fit for the conduct of its business.

Article VII

CORPORATE SEAL

The corporate seal shall be circular in form and have inscribed therein the following:—WOODS HOLE OCEANOGRAPHIC INSTITUTION MASSACHUSETTS 1930.

Article VIII

AMENDMENTS

These By-Laws may be from time to time in any manner altered by the Board of Trustees, at any meeting thereof, provided that the notice of the meeting states that an alternation of the By-Laws is to to be acted upon.

IV

REPORT OF THE TREASURER

June 1, 1933.

To the Members and Trustees of the Woods Hole Oceanographic Institution:

Gentlemen:

The accounts of the Treasurer through February 28, 1933, have been audited by Messrs. Seamans, Stetson & Tuttle, certified public accountants, and copies of their reports covering the three fiscal years are on file in the office of the Institution. Following are summaries of the various accounts:

PLANT ACCOUNT

Received from Rockefeller Foundation		$580,100.00
Interest on bank balances less Federal Tax on checks		542.13
Received from Carnegie Corporation		27,000.00
Transfers from Current Funds:		
For the year ending Feb. 28, 1931		5,072.32
For the year ending Feb. 29, 1932		8,386.94
For the year ending Feb. 28, 1933		7,734.21
Reserve for Depreciation		7,479.17
Atlantis construction	$218,674.47	
Atlantis equipment	25,991.95	
Land	27,072.32	
Laboratory construction	323,213.12	
Laboratory equipment	20,755.24	
Asterias construction and equipment	6,570.55	
Crew Room	557.95	
Books	6,000.00	
Cash Depreciation Fund	7,479.17	
	$636,314.77	$636,314.77

ENDOWMENT FUNDS

There was received from the Rockefeller Foundation for the General Endowment Fund	$1,000,000.00	
Less Loss on sale of Securities	1,305.50	$998,694.50
and from the same source for		
Endowment for Upkeep	$419,419.96	
Less Loss on sale of Securities	559.50	418,860.46

A reserve has been set up for amortization of Bond premiums, which now amounts to		$ 868.00
making a total of		$1,418,422.96
This is represented by Bonds of the cost of	$1,281,499.39	
and preferred Stocks at a cost of	134,793.75	
and Cash on hand	2,129.82	$1,418,422.96

a full list of the securities will be found in the Auditors' reports.

Interest and Dividends receivable from the Endowment Funds, if all securities pay in full, amount to	$64,310.00
During the year 1932–33, the actual amount received from the Endowment Funds, was	$63,818.92

RESERVE FUND

On March 14, 1931, the Institution received an anonymous gift of		$30,000.00
consisting of Cash in the amount of	$4,271.50	

and the balance in stocks.

This has been treated as a Reserve Fund to be used at the discretion of the Trustees.

GENERAL ACCOUNT

The current receipts and payments have been as follows:

For the period January 6, 1930, the date of its Charter, to February 28, 1931:

Receipts:

From the Rockefeller Foundation for Current Expenses for the year ended February 28, 1931	$50,000.00
Interest on Bank balances	94.87
	$50,094.87

Payments:

Scientific salaries		$ 6,500.00	
Administrative salaries, expenses, petty cash, etc.		3,323.82	
Organization expenses		3,245.27	
Insurance		830.32	
Travelling expenses		310.00	
Maintenance, Building and Grounds		269.55	
Interest on loans		84.72	
		$14,563.68	
Payment from Current Funds for Plant Assets:			
Library	$5,000.00		
Land	72.32	5,072.32	
Subscription to Trans. Arctic Submarine Expedition		25,000.00	$44,636.00
Balance on Hand, February 28, 1931			$ 5,458.87

For the period March 1, 1931 to February 29, 1932:

Income:		
From the Rockefeller Foundation for Current Expenses for the year ended February 29, 1932		$49,541.13
Interest and Dividends from Investments		35,452.05
Interest on Bank balances		68.76
Total Income		$85,061.94
Expense:		
Atlantis—Operation	$24,057.79	
Asterias—Operation	626.73	
Scientific salaries	22,114.58	
Laboratory supplies	1,096.74	
Administration expense	4,803.25	
Travelling expenses, etc.	1,336.08	
Office supplies	590.39	
Operation of truck	107.38	
Atlantis—Upkeep	10,486.00	
Upkeep of Building and Grounds	5,824.08	
Insurance	6,997.65	
Depreciation	3,000.00	
Amortization of Bond Premiums	434.00	
Payment to Bermuda Biological Station for Research	2,000.00	
Total Expense		$83,474.67
Excess of Current Income		$ 1,587.27

Of the above figures of expenditures $5,886.57 was credited to Plant Funds from Current Account, and later an additional amount of $2,500.37 was similarly treated.

Also a special gift of $10,000.00 was received for the Wilkins-Ellsworth Trans-Arctic Submarine Expedition and was turned over to them for the purchase of scientific instruments.

For the period from March 1, 1932 to February 28, 1933:

Income:		
From the Rockefeller Foundation for Current Expenses for the year ended February 28, 1933		$ 50,000.00
From Bingham Oceanographic Foundation of Yale University towards expense of Caribbean Voyage		1,500.00
Interest and Dividends from Investments		63,818.92
Interest on Bank balances		46.25
Total Income		$115,365.17
Expense:		
Upkeep of Plant:		
Upkeep of Building and Grounds	$ 6,958.47	
Atlantis—Upkeep	6,831.25	
Asterias—Upkeep	75.72	

Insurance	$ 7,004.13		
Reserved for Depreciation	6,000.00		
		$ 26,869.57	
Operating Expenses:			
Atlantis—Operation	$28,009.43		
Asterias—Operation	975.62		
Scientific salaries	34,944.34		
Scientific supplies and equipment	4,156.43		
Travelling expenses	710.88		
Grant to Bermuda Biological Station for Research	2,000.00		
Publications	678.08		
Administration	8,293.45		
Miscellaneous Expense	149.19		
Amortization of Bond Premiums	434.00	80,351.42	
Total Expense		$107,220.99	
Expenditure for Permanent Plant Additions:			
Additions to building	$ 250.00		
Additions to laboratory equipment	381.06		
Additions to *Atlantis* equipment	6,103.15		
Additions to library	1,000.00	7,734.21	
Total Expenditure			$114,955.20
Excess of Income			$ 409.97

The situation at the beginning of the present fiscal year, was as follows:

CURRENT ASSETS:		CURRENT LIABILITIES		
Cash	$3,581.69	Accounts—Payable		$4,010.62
		Accrued Payroll		548.50
Accounts—Receivable	46.60	Current Surplus and Reserve for "Working Balance":		
		Reserve for "Working Balance"	$5,000.00	
		Current Surplus (Deficit)	*5,930.83*	*930.83*
	$3,628.29			$3,628.29

Respectfully submitted,
Lawrason Riggs, Jr.,
Treasurer

V

REPORTS OF THE DIRECTOR

BY HENRY B. BIGELOW

FIRST ANNUAL REPORT FOR 1930

The concrete events that led up to the establishment of the Woods Hole Oceanographic Institution were, briefly: the appointment by the National Academy of Sciences of a Committee on Oceanography, the presentation by the latter, in the autumn of 1929, of a report on the scope and problems of oceanography and on the status of this science in North America; the adoption of this report by the Academy, with recommendation to the Rockefeller Foundation that an independent oceanographic institution be established on the east coast of North America; and the action of the Foundation in granting to its Executive Committee authority to aid in the construction and maintenance of such a station.

The Institution, incorporated under the laws of Massachusetts, received its charter on the sixth of January, 1930, for the following purposes as detailed in the Act of Incorporation:

> To prosecute the study of oceanography in all its branches; to maintain a laboratory or laboratories, together with boats and equipment and a school for instruction in oceanography and allied subjects; to accept and hold money, and property of any kind whatsoever, and wherever situated, and whether received through bequest, devise, gift or otherwise; and to apply from time to time and at any time to the purposes of the corporation, or to any of them, all or any part of the income and/or principal of any funds and/or property held by the corporation.

The first meeting for organization was held in New York on the fifteenth of January, when the following were chosen members of the corporation and as trustees to serve until the first annual meeting:

Thomas Barbour
Henry B. Bigelow
William Bowie
Newcomb Carlton
E. G. Conklin
Benjamin M. Duggar
Frank R. Lillie
John C. Merriam
Seward D. Prosser
Lawrason Riggs, Jr.
Harlow Shapley
T. Wayland Vaughan

The following officers were elected:

President, Frank R. Lillie
Treasurer, Lawrason Riggs, Jr.
Clerk, Henry B. Bigelow

Executive Committee:

Thomas Barbour
Henry B. Bigelow
E. G. Conklin
Frank R. Lillie
Lawrason Riggs, Jr.
Director, Henry B. Bigelow

Dr. Max Mason, President of the Rockefeller Foundation, informed this meeting that the Executive Committee of the Foundation was prepared to consider a formal request from the Woods Hole Oceanographic Institution for funds to carry out its building plan, to purchase equipment and to provide a permanent endowment for its support. An application to this effect was shortly forwarded to the Foundation and we were duly informed, on February 13th, that the latter had adopted the following resolves whereby the financial support of the Institution was assured:

> That the sum of Two Million dollars ($2,000,000), or so much thereof as may be necessary, be, and it is hereby, appropriated to the Woods Hole Oceanographic Institution, of which $1,000,000 shall be for Building and other construction, boats, equipment and endowment for upkeep of building payable as needed; and $1,000,000 shall be for Endowment of the station payable upon completion of the building.
>
> That the sum of Five Hundred Thousand Dollars ($500,000) or so much thereof as may be necessary, be, and it is hereby, appropriated to the Woods Hole Oceanographic Institution for Current Expenses over a period of ten years beginning approximately March 1, 1930, payments in any one year not to exceed $50,000.

Negotiations had already been opened with the Marine Biological Laboratory for the acquisition, from them, of the water-front site in Woods Hole that the president and director had selected as the most suitable for our laboratory building. The Biological Laboratory voted to transfer this site to the new Institution. On investigation, however, it was found that this could be done legally only through sale to the Oceanographic Institution at the fair market value. And as it had already been stated in the preliminary negotiations with the Rockefeller Foundation that no funds would be needed for the purchase of land, an application was made to the Carnegie Corporation for the necessary sum, $26,910. While awaiting their action, $5,000 was borrowed under authority of the trustees to make the initial payment, and title was passed from the Marine Biological Laboratory (previous owner) to the Woods Hole Oceanographic Institution on June 23d, 1930, for the land on which the laboratory building is now arising. The remainder of the price is to be paid in annual installments. The trustees consider that the Institution has been particularly fortunate in obtaining this plot of about 33,594 square feet of ground, for it combines the advantages of frontage upon deep water, a convenient location for a dock for our ship, an opportunity for expansion by further purchase in the future, and close vicinity to the laboratories already existing in Woods Hole. Under the zoning regulations of the Town of Falmouth, this site was in the industrial zone, but on February 12 the voters of Falmouth in Town Meeting voted to transfer it to the institutional zone.

Shortly after the organization meeting, the architectural firm of Coolidge, Shepley, Bulfinch and Abbott were engaged to prepare plans for the laboratory building. These called for a brick building, 135 feet long and 50 feet wide, of four stories and a basement, containing laboratories and research rooms to accommodate about 50 students and investigators, chart and reading rooms, refrigerating system, and the usual offices in the basement. The plans were approved by the trustees on May 3 and an expenditure not to exceed $400,000, was authorized for the construction of the laboratory, dock, float and sea wall. Competitive bids were called for, contracts let to the lowest bidders and construction commenced in July.

From the time when the prospects of the new Institution were first discussed in earnest, the trustees have appreciated that one of the most important things that it could do would be to operate a seagoing research ship of moderate size, with convenient living quarters, and equipped to carry on investigation at all depths in the various lines of sea science. No other American marine laboratory, independent of the government, is able to do this. A number of ships of various types were offered to the Institution, for purchase or as gifts. But none of them were satisfactory, either because of age, size, cost of upkeep, cost of conversion or because of other considerations. And at the May meeting, the Trustees authorized the Executive Committee to proceed with the planning and construction of a research ship at a cost not to exceed $175,000 including architects' fees and special hoisting machinery.

The firm of Owen & Minot, naval architects, who had already made preliminary sketches, were requested to complete the specifications.

The type of ship that would best fit our needs was the first question to be decided. Various scientific establishments in this country and abroad have most generously supplied us with plans and specifications of their ships, with full information as to their advantages and shortcomings. With this information at hand, and in view of the types of work that the Institution may be expected to carry out as well as of the amount of money that can be allotted to the operation of the ship, it was decided that a steel auxiliary, of about 350 gross

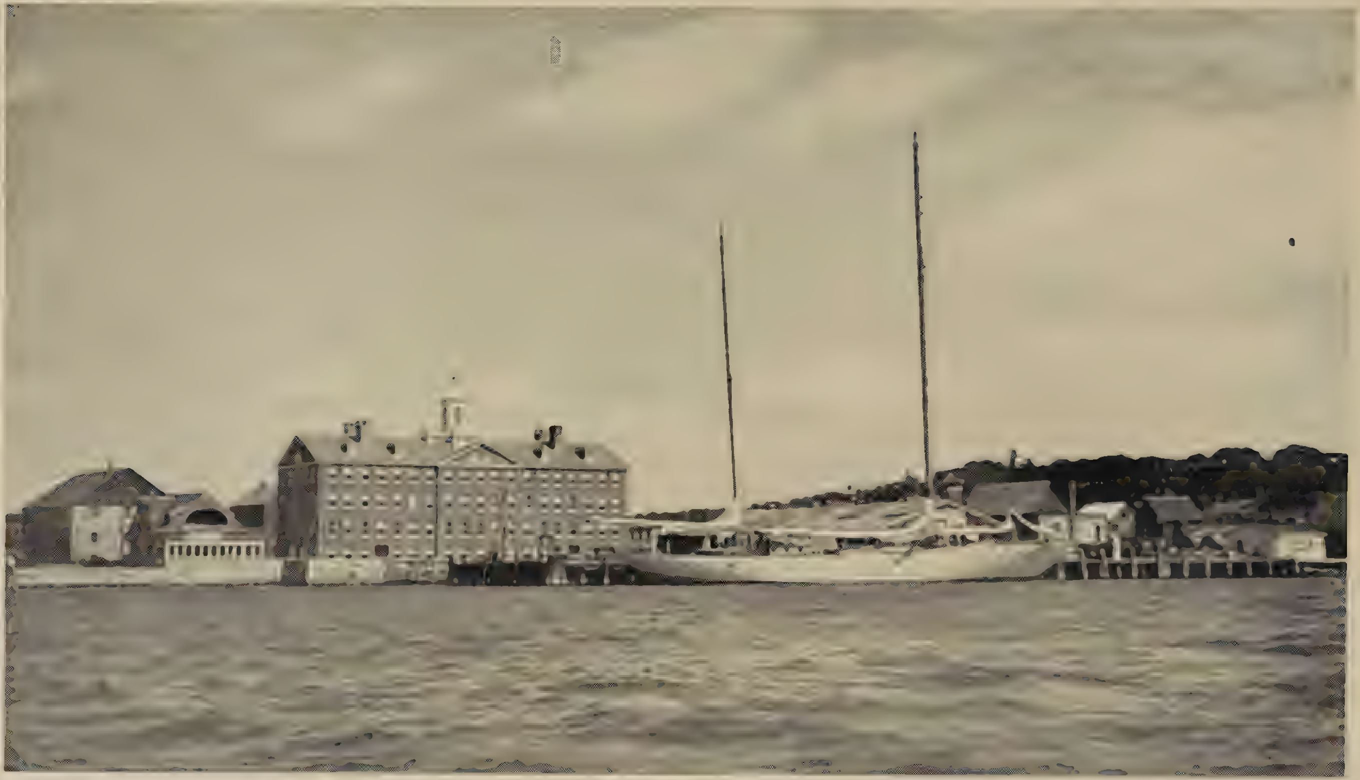


FIG. 1. Woods Hole Oceanographic Institution Building, Dock, and Research Vessel *Atlantis*

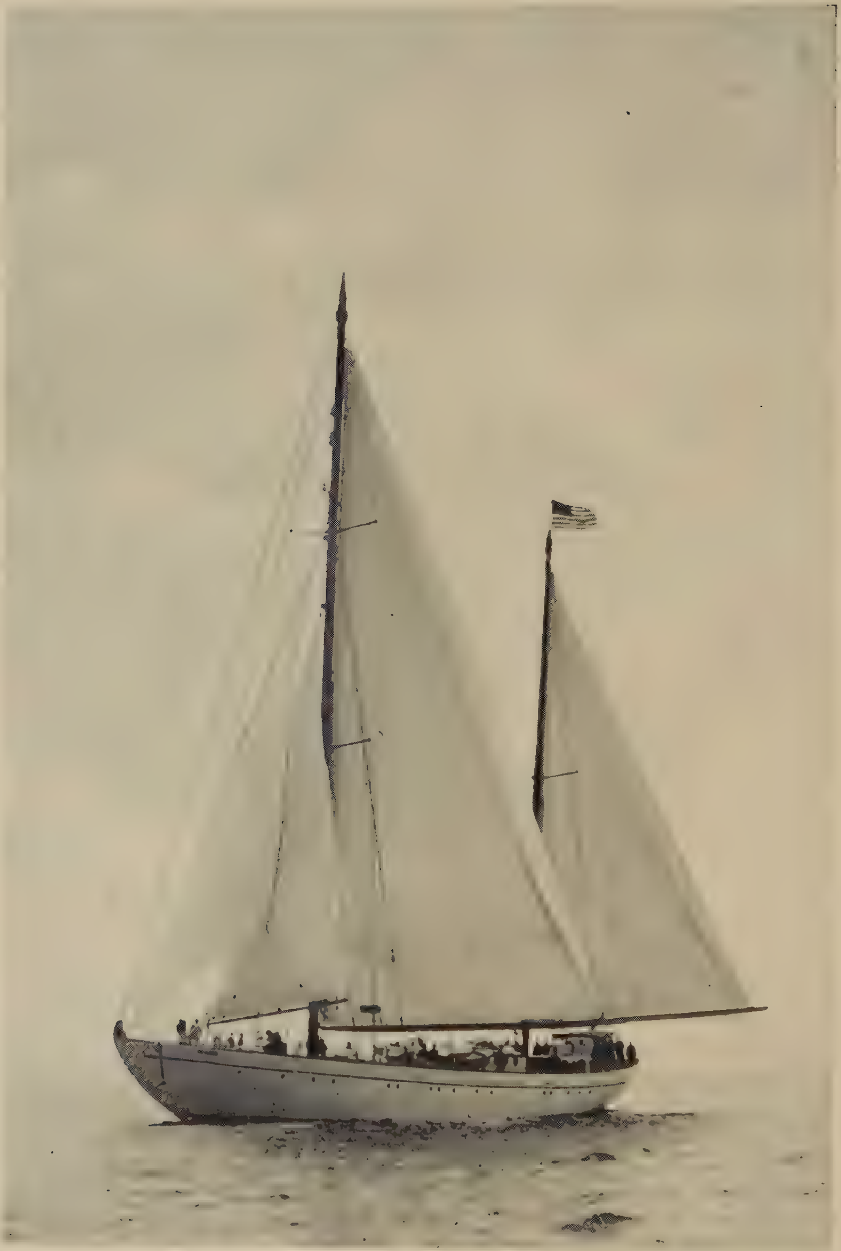


FIG. 2. *Atlantis*

tons, about 140 feet long, with hull of sailing ship type, would best serve our purposes. The plans, as finally developed, call for an overall length of 142 feet, a beam of 29 feet, a draft of 16 feet, a ketch rig spreading 7500 square feet of canvas, and a Diesel engine of 250–300 H.P. to develop a speed of about 8–9 knots. Two laboratories are provided for, one on the boat deck and one on the main deck. There are comfortable accommodations in staterooms for six scientists, besides the officers, with dormitory space for several students, and ample accommodations for the crew. The main hoisting winch is to be located in the lower hold, but controlled from the deck. On completion of the plans and specifications, competitive bids were requested from reputable shipyards in the United States, Germany, Scotland, Denmark, Sweden and Norway. The bid made by Burmeister & Wain Ltd., of Copenhagen being substantially the lowest, the contract was awarded to them, on vote of the Executive Committee under authority conferred by the trustees. The keel has been laid, and delivery is promised not later than June, 1931.

Two permanent appointments have been made to the staff in addition to the director: Mr. C. O. Iselin, to be general assistant and master of the research ship; Miss Virginia B. Walker, secretary to the director and administrative assistant. The selection of members of the scientific staff is now under discussion by the Executive Committee.

One of the inducements for the choice of Woods Hole as the site for the new Institution was the possibility of making use of the library of the Marine Biological Laboratory thus freeing us from the need of establishing a library of our own. The Biological Laboratory has decided to build up the oceanographic section of its library. And the trustees of the Institution have appropriated $5,000 for the current year for the purchase of books and subscriptions to journals. A list has been presented of desirable items not already in the library and most of this appropriation has already been spent or contracted for.

SECOND ANNUAL REPORT FOR 1931

The laboratory building, with pump house, dock, sea wall and float was completed during the winter and opened for occupancy June 15th. The expenditure for this part of our physical plant was not to exceed $400,000. The actual cost of construction complete was only $320,544.51 which includes all the furniture for the building for which we had originally expected to pay from the equipment appropriation. This saving resulted from the elimination of some items that seemed unnecessary, and from the favorable conditions in the building trades.

Attention is especially called to the excellent acoustic properties of the building. A departure has also been made from the procedure now usual in laboratory construction in the choice of the color for the wall surfaces.

The salt water pump system and cooling system for the aquaria tanks in the basement are both working well, and the heating system has proved adequate.

The arrangement of rooms within the building exactly follows the plans approved at the last annual meeting, except that a small bedroom has been added in the attic for the janitor. One of the large rooms in the basement has been arranged as a club room for the crews of "Atlantis" and "Asterias." A bath and shower have been installed, and furniture purchased, while a radio was presented by Mr. B. W. St. Clair.

Most of the time of the director and secretary during the late spring was devoted to ordering the chemical apparatus, glassware and the many other items that are called for in a laboratory in operation. None of the staff or associates who have been working in the building this summer have been seriously delayed on this score. A sum not to exceed $20,000 was voted for this purpose at the Trustees meeting on May 3d. The cost of the laboratory equipment to date has been about $17,673.03.

The original plans contemplated the installation of a gas machine, there being no other source of local supply. But the Town of Falmouth has recently granted a charter to the Buzzards Bay Gas Company, and we now receive gas from the street mains, which is much to our advantage.

The only thing that need be added further regarding our shore equipment is that the dock built to accommodate "Atlantis" is entirely satisfactory, and that the basin that it encloses has proved a convenient harbor for "Asterias."

One problem that faced us was the selection of a name for the research ship. At the meeting of the Executive Committee on September 27, 1930, it was voted

> that if Dr. Alexander Forbes is willing to transfer to the Institution the name "Atlantis" now borne by his schooner yacht, the research ship be named "Atlantis." Failing this, that she be named "Penikese," provided this name be not preoccupied.

Dr. Forbes very generously turned over the name "Atlantis" and rechristened his own yacht.

"Atlantis" was launched on December 31, 1930. I was able to inspect her in Copenhagen in March, when her interior fittings were sufficiently advanced to give a good idea of her finished state. It

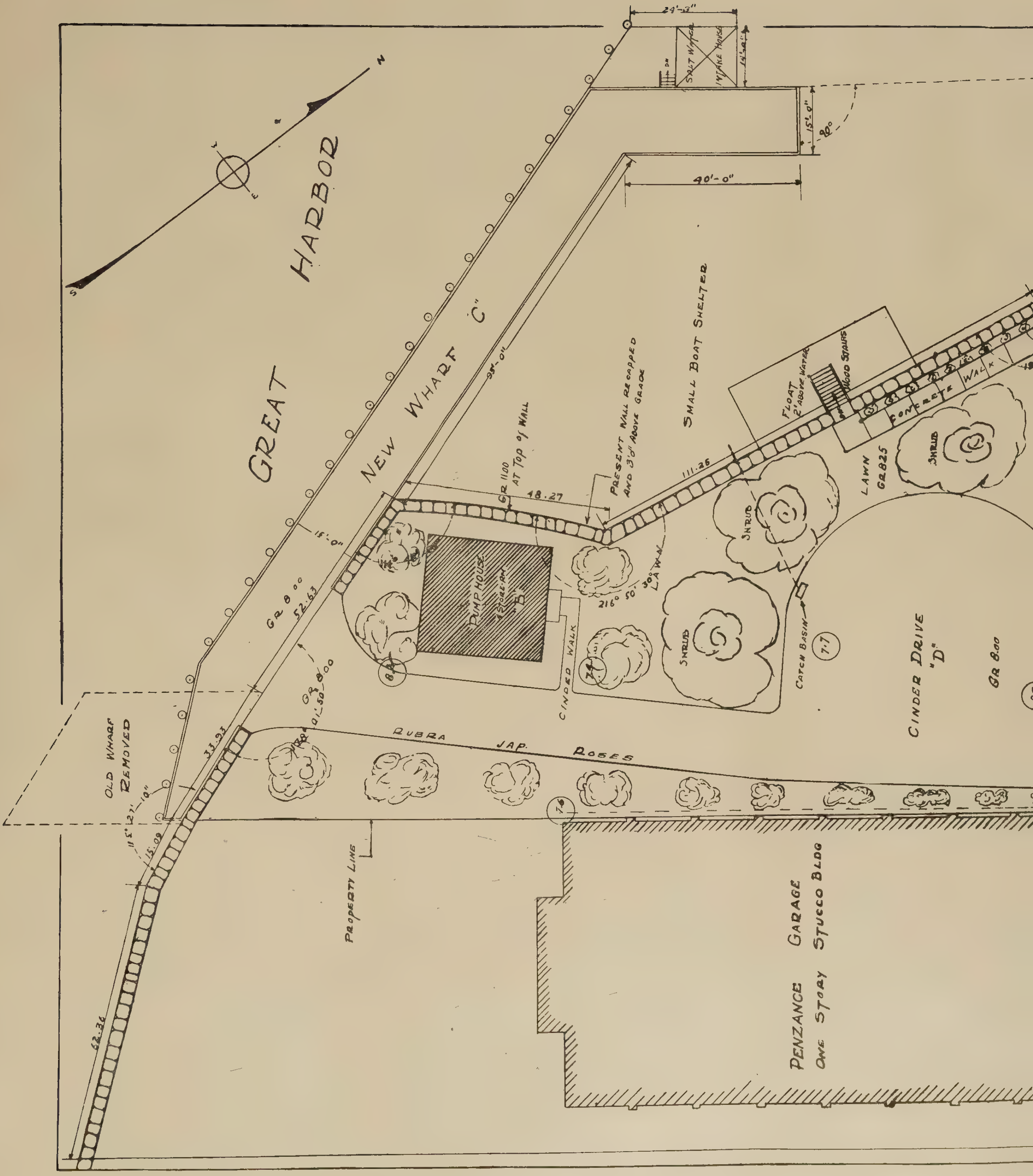
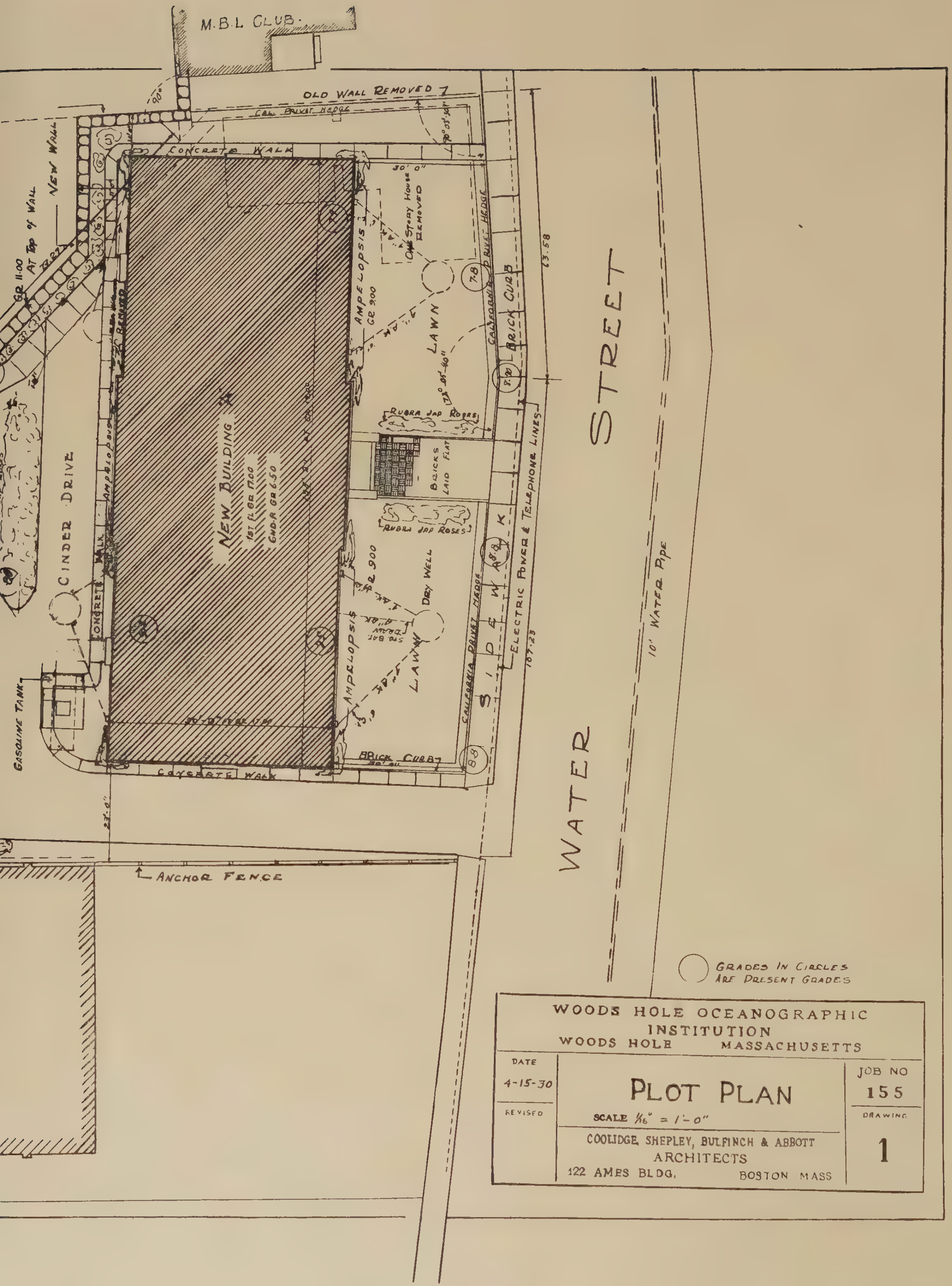

24'-3"
SALT WATER
INTAKE HOUSE
14'-8"
15'-0"
90°
40'-0"
N
S
GREAT HARBOR
NEW WHARF "C"
35'-0"
SMALL BOAT SHELTER
PRESENT WALL RECAPPED AND 3'0" ABOVE GRADE
GR 11.00 AT TOP of WALL
48.27
111.25
FLOAT 2' ABOVE WATER
WOOD STAIRS
CONCRETE WALK
LAWN GR 8.25
SHRUB
SHRUB
SHRUB
PUMP HOUSE
STORE-RM
"B"
CINDER WALK
LAWN
216° 50' 30°
CATCH BASIN
7.7
8.4
7.4
7.6
CINDER DRIVE "D"
GR 8.00
15'-0"
GR 8.00
52.63
GR 8.00
GR 10.50
RUBRA JAP. ROSES
OLD WHARF REMOVED
33.93
115° 27' 10"
15.09
PROPERTY LINE
PENZANCE GARAGE
ONE STORY STUCCO BLDG
62.36

FIG. 3. Plot of the Woods Hole C



M.B.L. CLUB.
OLD WALL REMOVED
CAL. PRIVET HEDGE
NEW WALL
GR 11.00 AT TOP OF WALL
CONCRETE WALK
30' 0"
ONE STORY HOUSE REMOVED
AMPELOPSIS GR 9.00
LAWN
CALIFORNIA PRIVET HEDGE
BRICK CURB
13.58
RUBRA JAP ROSES
BRICKS LAID FLAT
NEW BUILDING
1ST FLOOR 17.00
GND. GR 6.50
CINDER DRIVE
CONCRETE WALK
AMPELOPSIS
DRY WELL
GASOLINE TANK
SIDEWALK
ELECTRIC POWER & TELEPHONE LINES
107.23
WATER STREET
10" WATER PIPE
ANCHOR FENCE
23.0"
GRADES IN CIRCLES ARE PRESENT GRADES

WOODS HOLE OCEANOGRAPHIC INSTITUTION
WOODS HOLE MASSACHUSETTS

DATE 4-15-30
REVISED

PLOT PLAN
SCALE 1/16" = 1'-0"

COOLIDGE, SHEPLEY, BULFINCH & ABBOTT
ARCHITECTS
122 AMES BLDG. BOSTON MASS

JOB NO 155
DRAWING 1

anograhic Institution

speaks well for the builders that the only important suggestion that seemed necessary was for additional strengthening to the pilot house.

Mr. Iselin went to Copenhagen in April to supervise the last stages in construction. Mr. Minot, of Owen & Minot, our naval architects, was also in Copenhagen for the trials, which were held most successfully on the 18th of June, on which day the ship was accepted, payment made, and an American flag, presented to us by Mrs. Johannes Schmidt, wife of the famous Danish oceanographer, was hoisted over her. The ship has been classified *100 A 1 at Lloyds.

At the trials "Atlantis" developed a speed of 9.8 knots under power over a measured course instead of something like 8 knots which we expected, and on a lower fuel consumption than was promised.

At the meeting of May 3d, 1930, the sum of $175,000 was appropriated for the construction of the ship, including hoisting machinery, naval architects' and engineers' fees, and expenses of Lloyds Survey. At the meeting of September 27, an additional appropriation of $20,000 was made. The total cost of the construction of "Atlantis" including the items just stated is $208,129.77. That is to say, the cost exceeded the appropriation by $13,129.77.

The special hoisting machinery was manufactured by the Lidgerwood Winch Company of New York and shipped over to Copenhagen. Some of the cabin fittings were also sent across, the remainder purchased in Copenhagen. Most of the hydrographic apparatus for the ship, such as deep sea thermometers, water bottles, chemical apparatus, nets, meteorological apparatus and so forth was purchased in Norway, Germany and England. At the meeting of May 3d, a sum not to exceed $20,000 was voted for this purpose. The total cost of equipping the ship has been about $19,000.

We are indebted to the U. S. Coast and Geodetic Survey for the loan of a deep sea wire sounding apparatus, and to the U. S. Navy for the loan of a ship theodolite. The problem of sonic sounding gear for "Atlantis" has yet to be met, and this also applies to radio for sending messages.

"Atlantis" sailed from Copenhagen on July 2d, reaching Plymouth, England, July 7th, where she took on board such of the apparatus as had been ordered in London. She sailed from Plymouth July 16th, and arrived in Boston August 26th.

Shortly after "Atlantis" arrived in Woods Hole, in the autumn of 1931, she was docked at the Atlantic Iron Works, East Boston, for certain changes, the need of which became evident on her Atlantic crossing. The more important of these were: improvements to the plumbing system; installation of a new burner in the galley stove;

installation of 37 tons additional ballast; and some alterations to the rigging. The total cost was approximately $9,000, which was paid from current funds.

On December 13th, 1930, the Executive Committee voted not to exceed $7,000 for the construction of a 40 ft. launch for work near shore. The contract for this boat was let to the Casey Boat Building Company and she was delivered to us early in June, total cost $6,559.91. "Asterias," as she has been named, has proved extremely useful, providing for all the wants of the investigators at the laboratory during the past summer.

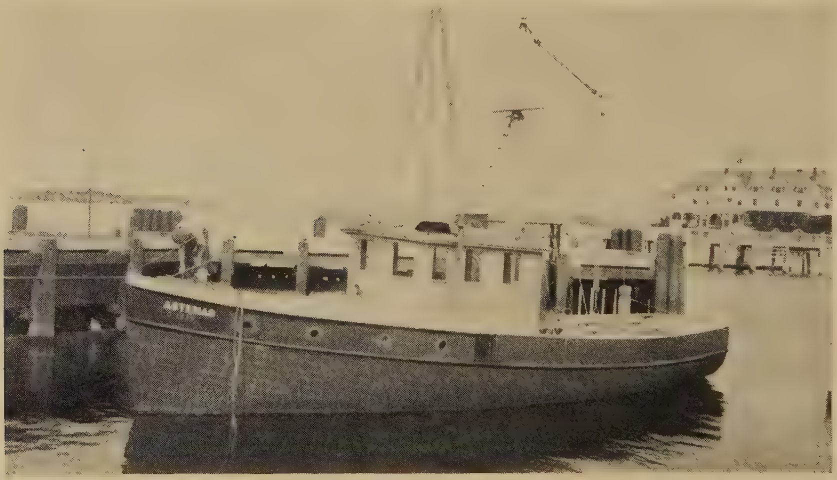


FIG. 4. *Asterias*

During the year, the following appointments were made to the scientific staff:

Alfred C. Redfield, Professor of Physiology, Harvard University, Senior Biologist.

Norris W. Rakestraw, Assistant Professor of Chemistry, Brown University, Research Associate in Physical Chemistry.

C. G. Rossby, Associate Professor of Meteorology, Massachusetts Institute of Technology, Research Associate in Dynamic Oceanography and Meteorology.

Henry C. Stetson, Assistant Curator of Palaeontology, Museum of Comparative Zoölogy, Research Associate in Submarine Geology.

Selman A. Waksman, Microbiologist, New Jersey Agricultural Experiment Station, Research Associate in Marine Bacteriology.

C. O. Iselin, Assistant Curator of Oceanography, Museum of Comparative Zoölogy, to be Research Associate in Physical Oceanography, as well as master of Research Ship "Atlantis."

Olav Mosby, Senior Physical Oceanographer, U. S. Coast Guard, Research Associate in Oceanography.

Captain Sir Hubert Wilkins, Research Associate in Oceanography.

An appropriation of $5,000 was provided for research assistants, of whom ten were appointed for varying terms. We have also had five visiting investigators. In all twenty-nine people (investigators and their assistants) worked in the laboratory during the past summer. The principal projects which it was planned to undertake during this first year of operation are listed in the first annual announcement; work has been started on all of these and in most cases appreciable advances have been made, as appears from the summaries of progress, forming the appendix to this report.

The scientific staff of "Atlantis," on her maiden voyage, consisted of Dr. Franz Zorell, who was enabled to take part through the cooperation of the Deutsche Seewarte in Hamburg, in charge of physical and chemical observations; Dr. George L. Clarke, Research Assistant in charge of Biological work; and of Mr. Raymond Montgomery, general scientific assistant. The scientific program consisted of physical and chemical observations at stations closely spaced along Long. 30° from Lat. 53° southward to a point about 200 miles south of the Azores, thence more widely spaced westward; experiments on light penetration with the light apparatus devised by Dr. Atkins of the Plymouth Laboratory; on the vertical distribution of plankton; and tows especially for the collection of eel larvae undertaken at the request of Dr. Johannes Schmidt.

Through the courtesy of the Marine Biological Laboratory, the staff and visitors to the Oceanographic Institution have enjoyed the full facilities of the library of the former.

Dr. C. J. Fish, Executive Secretary of the International Passamaquoddy Commission for Fisheries Investigations came to the Laboratory in October, to work there during the autumn and winter. In March I attended the meeting of the International Council for the Exploration of the Sea in Copenhagen, representing there not only this Institution, but the North American Council on Fisheries Investigations, and took part in most of the sectional meetings, through the courtesy of Mr. Henry G. Maurice, president of the Council.

At the final session of the latter, the following resolution was passed:

> The Council has heard with great interest the account given by Dr. H. B. Bigelow, Director of the Woods Hole Oceanographical Institution, of his plans for the investigations of the North Atlantic, particularly in its western part. It welcomes this important new development, and considers the moment opportune to enter into close co-operation both with the Woods Hole Oceanographic Institution and with the North American Council on Fisheries Investigations, believing that such co-operation will be fertile in results. It is of the opinion that effective co-operation can best be achieved by close personal touch between the workers and accordingly invites representatives of both sides to take part regularly in its deliberations, in order to arrive at unity of plan and methods, in the study of those fundamental problems which are similar or identical on both sides of the Atlantic.

The Council has extended an invitation to us to be represented at its future meetings.

THIRD ANNUAL REPORT FOR 1932

I

This report covers the first complete year of scientific activity.

Equipment

No important alterations or additions have been made to the buildings, nor have any extensive repairs been called for.

Experience on long cruises, and in heavy weather, has proved that "Atlantis" is an excellent sea boat; that no mistake was made in the selection of an auxiliary sailing ship for our particular work; and that her gear is well designed for modern deep-sea exploration. The fuel and water supply are ample for continuous passages up to two months in length. The fuel cost has also proven gratifyingly low; less than $15.00 per day on the average, depending, of course, on how much the engine is run.

On April 29, 1932, on her return from her long cruise in the Equatorial Atlantic, "Atlantis" struck a rock in New Bedford Harbor, with slight damage. She was docked in East Boston; and in carrying out repairs several plates were replaced, all expenses being borne by the underwriters. The period of this enforced docking, extended by a strike, gave opportunity for our own crew to clean and repaint parts of the tanks, and skin of the vessel, which are difficult of access.

I need only add further, with regard to the vessels, that all the ordinary work of maintenance, on both, has been carried out by their crews; so that both "Atlantis" and "Asterias" are in better condition today than when they were delivered by the builders.

Considerable additions have been made to the scientific equipment, the most important being a sonic sounding machine for "Atlantis." After investigation of various types, in use in this country and abroad, a Fathometer, manufactured by the Submarine Signal Company, of Boston, was purchased, and installed in October. It was given its sea trials shortly after; and has been in regular use on the ship since then. Up to December 31, 1932, 422 sonic soundings had been taken with it, in depths greater than 200 meters.

II

Library

The arrangement by which workers at the Oceanographic Institution enjoy the facilities of the library of the Marine Biological Laboratory works well. During the current fiscal year $1,000 has been allocated to purchase of books and subscriptions to serials; including a small collection of text books, frequently needed, that are to be kept permanently in our own reading room, for the convenience of the staff.

III

Personnel

During the year the scientific personnel has been placed on a more permanent basis, through additions and reappointments. The present staff (December 31, 1932) is listed on p. 29.

The budget for the current fiscal year included an item of $4,000 for stipends for research assistants. The following sixteen appointments were made, for terms ranging from three to six months:—A. E. Alexander, Eliot F. Beach, Miss E. May Bixby, Frank A. Brown, Jr., Miss Mildred H. Campbell, Erik F. B. Fries, E. Monroe Harwood, Stephen Lichtblau, Frank B. Lutz, Raymond Montgomery, C. E. Renn, Raymond W. Root, Miss Mary Sears, Mrs. Gladys Seiwell, John H. Welsh and Miss Carola Whitman.

During the past year, the following fourteen visiting investigators have worked in our laboratory:—Dr. N. A. Borodin, Dr. Cornelia L. Carey, Mr. John S. Colman, Mr. Gardner Emmons, Dr. C. J. Fish, Dr. H. H. Gran, Dr. Arda A. Green, Dr. Margaret Hotchkiss, Dr. Martin Johnson, Dr. Roderick Macdonald, Dr. P. H. Mitchell, Miss Louise Palmer, Dr. Leif Størmer and Dr. C. B. Wilson.

Staff, visiting investigators and research assistants represent the following eighteen institutions:—Barnard College, Brown University, Cambridge University, England, Carnegie Institution, City College of New York, Clark University, Harvard University, International Passamaquoddy Commission for Fisheries Investigations, Massachusetts Institute of Technology, Massachusetts State

Teachers College, New York Homeopathic College, New York University, University of Oslo, Radcliffe College, University of Toronto, Tufts College, University of Washington, U. S. Bureau of Fisheries and U. S. Coast Guard.

Professor H. H. Gran, of the University of Oslo, Norway, is visiting the Institution, by invitation, under item provided in the budget.

The list also includes five technicians and other laboratory assistants.

The major projects, on which the staff and visiting investigators have been engaged, are summarized on Pages 10–12 of the annual announcement for the current year.

In May, Mr. W. C. Schroeder, formerly of the U. S. Bureau of Fisheries, was appointed "business manager" of the Institution.

On June 1st Captain Frederick S. McMurray was given a temporary appointment as master of "Atlantis" in order that Mr. Iselin might be relieved of the responsibilities of command to devote himself primarily to his researches in physical oceanography. Captain McMurray's appointment was made permanent later on October 10th.

Mr. William Warner was again in charge of "Asterias," assisted by an amateur crew.

IV

Publication

Consideration of facilities at present existing in America for publications in oceanography, and of the lines of work now being done in the Institution, lead to the conclusion that our original intent to start a journal to cover the whole field of oceanography would not adequately meet the situation. To care for papers in physical oceanography, an arrangement has been made for the joint publication, by the Institution, and the Massachusetts Institute of Technology, of a serial, entitled "Papers in Physical Oceanography and Meteorology," in continuation of the Meteorological serial now published by the Massachusetts Institute of Technology. The scientific results of the "Nautilus" Expedition, to form the initial volume of the new journal, are now in press. Arrangements for the publication of work in oceanic biology and chemistry are now under consideration.

Several reports on work done last year at the Institution, listed below, have either appeared or are in process of publication. We have contributed to the cost of one of these, by Professor Rossby.

V

Co-operation with other Institutions

The measures of economy put in force by the Federal Government have made it necessary for the Bureau of Fisheries to lay up the Fisheries' steamer "Albatross II." To provide for the continuation of the Bureau's investigation of the biology of the mackerel, "Atlantis" was sent out on two short cruises during July, for this work, in charge of Mr. O. E. Sette, of the Bureau. And tentative plans have been made for similar cruises, next spring, to map the distribution of eggs and larvae of the haddock.

Dr. C. J. Fish, Dr. Martin Johnson, Dr. E. W. Watson and Mr. Trygve Braarud of the International Passamaquoddy Commission came to the laboratory in October to spend the winter.

On the other hand a recording tide gauge has been loaned to the Institution by the U. S. Coast & Geodetic Survey. The U. S. Lighthouse Service has made it possible for us to obtain evaporation measurements at Nantucket Lightship; the Canadian Meteorological Service has arranged for pilot balloon observations at Sable Island; while the U. S. Navy Department, the U. S. Coast Guard, the U. S. Bureau of Fisheries, the U. S. Coast & Geodetic Survey, and the Marine Biological Laboratory have aided our activities in many ways.

The staff of the Institution also takes an active part in the activities of the following bodies: International Ice Patrol; North American Council for Fisheries Investigation; International Passamaquoddy Commission for Fisheries Investigation and the Marine Biological Laboratory. I was not able to attend the meeting of the International Council for the Exploration of the Sea last spring, but hope to attend the next meeting.

VI

Field Work

"Atlantis" carried out the following Cruises from July 15, 1931 to Dec. 31, 1932:—

Cruise 1. Plymouth, England, to Boston
2. Woods Hole, Nantucket Shoals
3. Cape Cod to Cape Hatteras
4. To the southward of Woods Hole
5. Bermuda, Cape Hatteras, Nova Scotia
6. Equatorial Atlantic

7. Continental shelf, between Cape Cod and Chesapeake Bay
8. Gulf of Maine
9. New York to Gulf of Maine
10. Gulf of Maine and Georges Bank
11. Nova Scotia, Bermuda, Chesapeake Bay
12. Gulf of Maine
13. To the southward of Woods Hole
14. Cape Hatteras to Bermuda, Chesapeake Bay to Cape Cod

Total days at sea	251
Total number of stations	450
Length of scientific sections	14,100 miles
Temperature and salinity observations	3,923
Oxygen determinations	2,370
Alkalinity determinations	2,132
Phosphate determinations	2,591
PO_4	750
Samples for Nitrate and Nitrite	256
Evaporation measurements	160
Pilot Balloon ascents	58
Light intensity determinations	23
Hauls for vertical distribution of plankton	49 (275 samples)
Tows for larval eels	33
Deep sea tows, especially for fishes	17
Oblique plankton hauls, deep	57
Vertical plankton hauls, 3000 meters	6
Plankton hauls for U. S. Bureau of Fisheries	110

"Asterias" was used from the middle of June until October, for trips in the vicinity of Woods Hole, the continental shelf to the southward, and around Cape Cod. Lines of bottom samples were taken off Block Island, No Mans Land and Cape Cod, for Mr. Stetson; in most cases out to the 100 fathom curve. Water samples for chemical analysis and temperatures were taken on all the lines. Some analyses were made on board; but this is difficult on so small a boat. Water samples and temperatures were also taken in the neighboring bays. Long cores (3–4 feet) of the bottom mud were collected at a dozen stations. Water and mud samples have been taken at a large number of stations, for bacteriological study. Plankton tows were taken in Vineyard Sound for Dr. Clarke and Miss Campbell; visits were made weekly to the fish traps in Menemsha bight, to obtain squid, and cer-

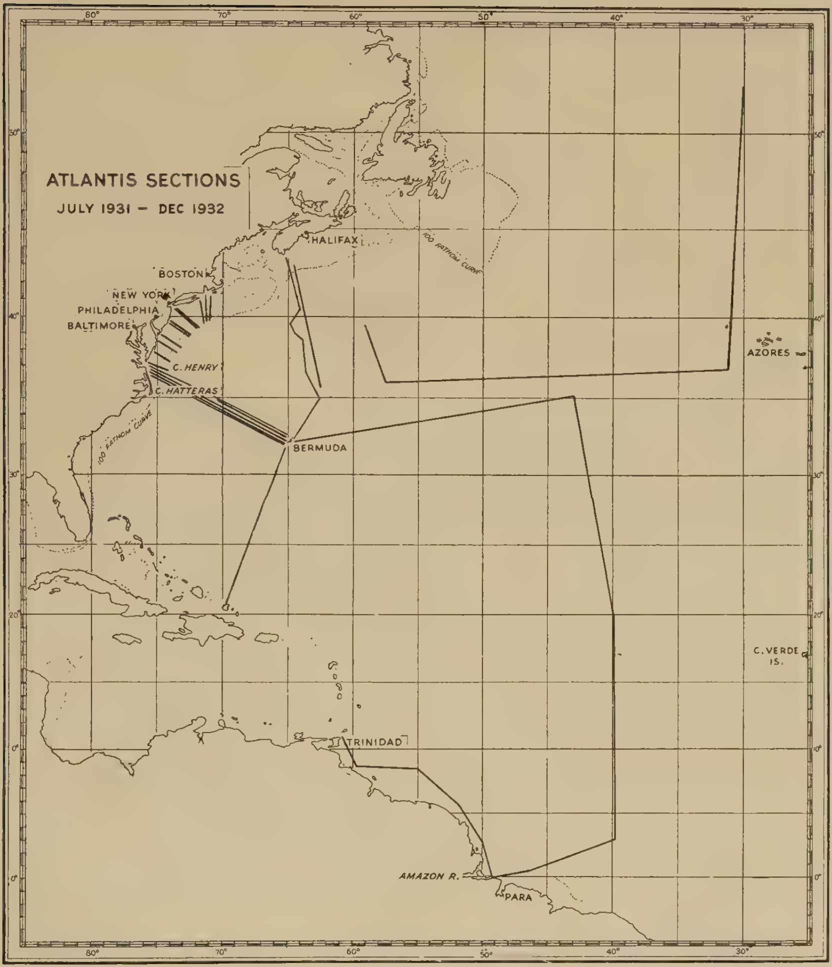

ATLANTIS SECTIONS
JULY 1931 – DEC 1932
HALIFAX
BOSTON
NEW YORK
PHILADELPHIA
BALTIMORE
C. HENRY
C. HATTERAS
100 FATHOM CURVE
100 FATHOM CURVE
BERMUDA
AZORES
C. VERDE IS.
TRINIDAD
AMAZON R.
PARA
80°
70°
60°
50°
40°
30°
50°
40°
30°
20°
10°
0°

FIG. 5. Profiles of *Atlantis* Cruises during 1931 and 1932

tain fishes, for Dr. Redfield and his associates; and more than 1,000 horseshoe crabs were collected, also for Dr. Redfield.

VII

Scientific Program

The major projects on which the staff and visiting investigators have been engaged during the year are listed in the annual announcement. And following the procedure introduced last year, brief summaries of progress are included in this report.

VI

STAFF

(As of December 31, 1932)

The staff consists of permanent scientific members and of research associates appointed for definite terms. The present personnel is as follows:

HENRY B. BIGELOW, Professor of Zoölogy, Harvard University, Director
GEORGE L. CLARKE, Tutor and Instructor, Harvard University, Junior Biologist
C. O. ISELIN, II, Assistant Curator of Oceanography, Museum of Comparative Zoölogy, Research Associate in Physical Oceanography
A. E. PARR, Director, Bingham Oceanographic Foundation, Yale University, Research Associate in Oceanography
NORRIS W. RAKESTRAW, Associate Professor of Chemistry, Brown University, Research Associate in Physical Chemistry
ALFRED C. REDFIELD, Professor of Physiology, Harvard University, Senior Biologist
HERBERT W. REUSZER, Instructor, Rutgers University, Junior Marine Bacteriologist
C. G. ROSSBY, Professor of Meteorology, Massachusetts Institute of Technology, Oceanographer
H. R. SEIWELL, Investigator in Oceanography
HENRY C. STETSON, Assistant Curator of Paleontology, Museum of Comparative Zoölogy, Research Associate in Submarine Geology
SELMAN A. WAKSMAN, Microbiologist, New Jersey Agricultural Experiment Station, Marine Bacteriologist
CAPTAIN SIR HUBERT WILKINS, Research Associate in Oceanography

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WILLIAM C. SCHROEDER, Business Manager
MISS VIRGINIA B. WALKER, Secretary and Administrative Assistant
WILLIAM SCHROEDER, Superintendent of Buildings and Grounds

VII

MEMBERS OF THE CORPORATION

(As of December 31, 1932)

Charles Francis Adams, Navy Department, Washington, D.C.
Thomas Barbour, Museum of Comparative Zoölogy, Cambridge, Mass.
William Bowie, U. S. Coast & Geodetic Survey, Washington, D.C.
Isaiah Bowman, American Geographical Society, New York, N.Y.
Newcomb Carlton, New York, N.Y.
E. G. Conklin, Princeton University
Benjamin M. Duggar, University of Wisconsin
John A. Fleming, Carnegie Institution, Washington, D.C.
Alexander Forbes, Harvard Medical School
Ross G. Harrison, Yale University
A. G. Huntsman, University of Toronto
Frank R. Lillie, University of Chicago
G. W. Littlehales, Hydrographic Office, Washington, D.C.
Alfred F. Loomis, Tuxedo Park, N.Y.
John C. Merriman, Carnegie Institution, Washington, D.C.
T. H. Morgan, California Institute of Technology
R. S. Patton, U. S. Coast & Geodetic Survey, Washington, D.C.
Seward D. Prosser, New York, N.Y.
Elihu Root, Jr., New York, N.Y.
R. P. Scripps, Ridgefield, Conn.
Harlow Shapley, Harvard University
B. W. St. Clair, West Lynn, Mass.
T. Wayland Vaughan, Scripps Institution of Oceanography, La Jolla, Calif.

APPENDIX A

Reports of progress by investigators working at the Institution during 1931.

PHYSICAL STUDY OF THE SHELF WATERS, CAPE COD TO CHESAPEAKE BAY

H. B. BIGELOW

Horizontal projections for temperature and salinity at various depth levels, have been prepared for most of the cruises of the "Albatross II" in the sector, the dynamic contours have been calculated for several of the months, and a general report is in course of preparation.

INVESTIGATION OF THE PHENOMENA OF RESUSCITATION OF FISHES, AFTER FREEZING

N. A. BORODIN

Studies were made of the problem of the "Anabiosis" of several species of fishes, or their recovery after being frozen. The duration of exposure and the lowest temperature which each species can withstand were determined.

STUDIES OF THERMAL DISTRIBUTION AT THE SURFACE OF THE NORTHWESTERN ATLANTIC, BASED ON THERMOGRAPH READINGS ON COMMERCIAL STEAMSHIP ROUTES

C. F. BROOKS, P. E. CHURCH AND K. B. ALLAN

Thirty-seven synoptic charts of surface temperatures of the Atlantic, west of 62° W. Long. and north of 30° N. Lat. have been made from data automatically recorded by thermographs installed on commercial vessels, which cross the area at regular intervals. The period of record is from January 1929 to May 1931. A detailed report of the variations and fluctuations of the temperatures is in process of preparation.

VERTICAL DISTRIBUTION AND VERTICAL MIGRATIONS OF PLANKTON, IN THE ATLANTIC BASIN, IN RELATION TO PENETRATION OF LIGHT

GEORGE L. CLARKE

a) Collections to determine the vertical distribution of zoöplankton were made at 10 "Atlantis" stations. Five 75-cm. tow nets attached at intervals to the main cable and opened and closed simultaneously were used for this purpose. The series of observations were repeated at different hours of the day to study the diurnal vertical migration of the plankton.

b) Collections to determine the vertical distribution of phytoplankton were made at one "Atlantis" station.

c) Measurements of the intensity of daylight falling on deck and penetrating to depths down to 175 m. beneath the sea surface were made at 13 stations, and at different times of day under various conditions. The photo-electric method developed by Poole and Atkins was used. The observations were made at the same time as the plankton hauls in order that the relation between the conditions of submarine illumination and the changes in the vertical distribution of the plankton could be studied.

STUDY OF THE CIRCULATORY AND THERMAL RESULTS OF THE PUMPING EFFECT OF THE TIDES OVER SUBMARINE SLOPES

GARDNER EMMONS

In connection with a study of thermal effects produced by the tide flowing over the slope between Nantucket Shoals and the deep waters of the Gulf of Maine, hydrographic observations were made from an anchored boat at two stations to the eastward of Pollock Rip Lightvessel. Each station was occupied for a period of 12 hours and 50 minutes through one complete tidal cycle. Temperatures and water samples from various depths were taken at intervals of one hour. In addition two hydrographic sections running northeast from Great Round Shoal whistling buoy were made on opposite stages of the tide.

SUMMARY OF HYDROGRAPHIC OBSERVATIONS TAKEN FROM "ATLANTIS" ON VOYAGE FROM PLYMOUTH, ENGLAND, TO WOODS HOLE

COLUMBUS ISELIN

July 26–Aug. 6, 25 stations down to 2000 meters between Lat. 52° N. Long. 30° W. and Lat. 37° N. Long. 32° W.

Aug. 9–Aug. 18, 5 stations down to 4000 meters between Lat. 37° N. Long. 32° W. and Lat. 37° N. Long. 57° W.

Aug. 18–Aug. 20, 8 stations down to 3000 meters between Lat. 37° N. Long. 57° W. and Lat. 39° N. Long. 59° W.

The temperature observations totaled 454 and about the same number of water samples were analyzed for both salinity and oxygen.

STUDIES OF THE OCEANOGRAPHY OF THE GRAND BANKS REGION IN THE SPRING OF 1931 BASED ON DATA OBTAINED BY THE ICE PATROL

OLAV MOSBY

The investigations accomplished this summer in the northwestern Atlantic consist of 184 oceanographic stations arranged in 14 different sections across the Labrador current between 40° N. and 61° N., one section between Resolution Island and Ivigtut, one section between Cape Farewell and Straits of Belle Isle, and observations in the middle of the sea between Labrador and Greenland.

STUDIES OF THE PHYSICAL CHEMISTRY OF SEA WATER

NORRIS W. RAKESTRAW

The chemical work carried on at the Woods Hole Oceanographic Institution during the summer of 1931 consisted for the most part of a general chemical survey of the waters of the region, with respect to the following factors: chloride, phosphate, nitrate, nitrite and oxygen (on a few samples).

Thirty-eight stations were visited at least once during the summer, and a few stations were visited twice. Samples were of course taken at various depths at each station. The results, particularly complete in the case of phosphate, nitrite and nitrate, give a fair picture of the conditions in this neighborhood, from the shore out as far as the edge of the continental shelf. Some interesting variations in distribution were observed and several new opportunities for investigation were revealed.

Some progress was also made in the perfection of methods for the determination of arsenic, iron and aluminum in the sea water. This field will be further developed in the future.

Samples were also collected from particular stations upon which we hope to carry out complete analyses during the next year.

STUDIES ON THE PHYSICAL CHEMISTRY OF RESPIRATORY PROTEINS

ALFRED C. REDFIELD

During the past summer equipment was assembled for the study of the effect of temperature upon the equilibrium of the blood of marine invertebrates with oxygen. A complete series of data has been obtained upon the blood of the horseshoe crab at temperatures varying between 5°C. and 44°C.

Material has also been collected to be used in studies of the chemistry of hemocyanin now in progress in the laboratories of Prof. J. B. Conant at Harvard, Prof. J. Barcroft at Cambridge University, and Prof. E. Philippi at the University of Innsbruck.

STUDIES OF THE STRESS EXERTED BY THE WIND AS A PROPULSIVE FORCE ON THE SURFACE OF THE SEA, AND OF THE EVAPORATION FROM THE LATTER

C. G. ROSSBY

To calculate the tangential stress of the wind along the ocean surface about sixty pilot balloon observations were made on two trips of the U. S. Bureau of Fisheries' steamer "Albatross II," to Georges Bank and the Gulf of Maine. Through the courtesy of Dr. J. Patterson, Director of the Canadian Meteorological Service, regular pilot balloon observations from Sable Island have been made available to us for the same purpose.

Some preliminary stress calculations were made from the numerous pilot balloon observations collected by staff members of the Deutsche Seewarte on trips between Europe and Central or South America.

Regular evaporation measurements were carried out during the home cruise of the "Atlantis." Arrangements have now been completed to obtain similar measurements from the Nantucket Lightship during the winter of 1931–1932.

CHEMICAL STUDIES OF SUBMARINE SEDIMENTS

H. R. SEIWELL

A critical study of certain physical-chemical characteristics of sediments of the coastal shelf was undertaken with the object of evaluating the relative importance of the sea bottom as a source of supply to the chemical fertility of the waters of the Woods Hole region. The study was limited to observations on the phosphorus cycle.

Two types of phosphorus were determined, the available or water soluble phosphate and the potential or organically combined phosphate. Quantitative estimations of organic matter and complete mechanical analyses were made as parallel determinations.

Sixty-three bottom samples from as many localities on the coastal shelf were collected and analyzed. A special technic for a critical analysis of these sediments has been developed. Methods for the colorimetric estimation of both soluble and insoluble phosphate of sediments have been improved and a method for determining the exact weight of the various fractions of sediments after their contamination with sea salts has been developed.

INVESTIGATION OF THE DISTRIBUTION OF MARINE SEDIMENTS FROM THE SHORE LINE OUT TO THE CONTINENTAL SHELF

HENRY C. STETSON

A new type of bottom sampler devised by C. Iselin was used. This consists of a circular tube rotated by a gear, driven by a drum upon which flexible wire has been wound. It is released by the usual type of tripper, and the wire on the drum, which now becomes the hoisting line, is completely unwound before the dredge is lifted from the bottom. The main advantages over other types of surface samplers are: the cutting tube is self closing so that no particle of sediment can wash out on the trip to the surface; a uniform sample is secured at each station as the cutter always scoops to the same depth below the surface.

Two lines of bottom samples (about 70 in each) were taken off Martha's Vineyard and Block Island, from the shore out to the 100 fathom curve. Two short lines of about 12 samples each were taken over the edge of the shelf off Martha's Vineyard from the 66 fathom to the 300 fathom contour. Two lines of 25 samples each were run off Truro and Highland Light, Cape Cod, from the shore out to the 100 fathom contour.

The coarse fraction of every sample has been worked up, and the fine fraction has been prepared for the hydrometer tests.

Some experiments were made with pans for collecting sediment, which opened up an interesting problem, for it appears that in ordinary weather the character of the sediment, moving over the bottom, is very different from the character of the latter.

Eighteen long cores were taken and sent to Dr. J. A. Cushman, to be examined for the contained Foraminifera.

Several gallons of gelatinous material, from the superficial layer of the sediment, were collected for Prof. J. B. Conant, for chemical study.

STUDIES IN MARINE BACTERIOLOGY

SELMAN A. WAKSMAN, ASSISTED BY H. W. REUSZER

The work in marine bacteriology has dealt with the determination of the total numbers of bacteria present in sea water and bottom deposits, and with the occurrence of bacteria capable of carrying on specific physiological processes.

The methods of approach have been:

1. The use of synthetic media.
2. Utilization of specific silica-gel media for determining the presence of bacteria active in specific processes.
3. Employment of enrichment culture media for studying marine bacterial processes under controlled conditions.

Results so far obtained are as follows:

1. The numbers of bacteria in the vicinity of Woods Hole range from 55,000 to 466,000 aerobic, and 4,000 to 40,000 anaerobic per cubic centimeter of sea water; from 14,000 to 430,000 aerobic and 3,300 to 40,000 anaerobic per gram of mud.
2. At least four distinct types of bacteria capable of decomposing cellulose have been isolated from sea water and from mud.

Most of these cellulose-decomposing bacteria are capable of liquefying agar and bring about the hydrolysis of the agar. Further studies are being undertaken on the relation between this process and the fixation of nitrogen by bacteria in the sea water.

3. Agar-liquefying bacteria have been isolated, but in smaller numbers than have been found in brackish situations in the Bahamas.
4. The presence of nitrogen-fixing bacteria in the sea water has been demonstrated by chemical determination of the amount of nitrogen fixed by them in laboratory experiments. The cultures seem to comprise both aerobic forms, of the Azotobacter type, and anaerobic, gas forming bacteria of the Clostridium type.

Studies of the rôle of bacteria in the decomposition of organic substance, and in the liberation of inorganic nutrients, in sea water and sea bottom deposits, are in progress.

INVESTIGATIONS ON THE NATURE OF THE PHYSIOLOGICAL FACTORS CONTROLLING DAILY RHYTHMS AND PHOTOTROPIC RESPONSES IN MARINE ORGANISMS

J. H. WELSH

The work consisted of a study of light intensity and temperature as factors controlling the rate of swimming of crustacean larvae. The larvae of the mussel crab, Pinnotheres, were selected as being easily obtained in large quantities throughout the season. The experiments were all carried on in the dark room under carefully controlled conditions and results were obtained which show that free-swimming organisms such as crab larvae show a change of rate with change of light intensity, which obeys the Bunsen-Roscoe Law.

APPENDIX B

Report of progress by investigators working at the Institution during 1932.

PHYSICAL OCEANOGRAPHY OF THE WATERS ON THE CONTINENTAL SHELF, CAPE COD TO CHESAPEAKE BAY

HENRY B. BIGELOW

During the year 1932, "Atlantis" cruised the area in June, September and December, adding serial observations at 68 stations to the data previously obtained. The report on the cycle of temperature is now nearly ready for publication.

INVESTIGATION OF THE PHENOMENON OF THE "ANABIOSIS" OR RESUSCITATION OF FISHES, AFTER FREEZING

N. A. BORODIN

Continuing the investigation started in 1931, 151 experiments on freezing fishes and efforts to revive them were made. There were about as many positive results as negative.

Besides this, 15 observations were made on the temperature of the body of 7 species of fish during the process of freezing.

Most of the experiments were made in dry cold air. Attempts to revive fish after freezing in water invariably gave negative results. The temperature of freezing and the duration of exposure are both of great importance. In most cases a temperature below −18°C. and a duration of one hour or more were fatal to all the species of fishes used in our experiments. There is, however, a considerable variability in the resistance of different kinds of fish. Fish that have recovered after being frozen may live for weeks.

Fish frozen to different degrees may be classified as either stiff, soft-frozen or hard-frozen. Histological examination has shown that the blood vessels of fish frozen only to stiffness, or soft-frozen, still bleed and there are no crystals of ice in muscles or intestines, while in hard-frozen fish the muscles, blood vessels and intestines contain ice-crystals. Hard freezing of the body of a fish is always accompanied by a complete freezing of the heart, which stops beating, and such a fish cannot recover.

The observations show that the temperature of the body decreases very slowly during the process of freezing: 45 minutes was needed to lower the temperature from +22°C. to −1.8°C. Only if the temperature of the fish remains above −0.6°C. or −1°C. do they recover. This body temperature seems to be the limit of life in the fish experimented upon.

Some supplementary experiments were made in the laboratory of the Museum of Comparative Zoölogy with Alaska blackfish (*Dallia pectoralis* Bean) known to be very hardy in regard to frost. Our experiments have shown that this fish can recover after being frozen at a temperature of −20°C. for 40 minutes, i.e., it is the hardiest fish of all so far experimented upon. But it did not recover from freezing in a block of ice. The temperature of its body was found to be +22°C. while the temperature of water in which it was kept was only +6.5°C.

STUDIES OF THERMAL DISTRIBUTION AT THE SURFACE OF THE WESTERN NORTH ATLANTIC, BASED ON THERMOGRAPH RECORDS ON COMMERCIAL STEAMSHIP ROUTES

C. F. BROOKS AND E. M. HARWOOD, JR.

Thirty-three weekly synoptic charts of surface temperatures, in addition to the thirty-seven previously made, were drawn for the surface of the Atlantic, west of the longitude of Bermuda to the Straits of Florida, and north of the latitude of San Juan, P.R., from the thermograph records taken on commercial steamships, which cross the area at frequent intervals. The charts now include the period from January, 1929, to April, 1932. Tabulation of the temperatures by weeks has been completed for most of the steamship lines, and for the New York-Puerto Rico line the tabulations have been graphed by major temperature divisions from October, 1926, to April, 1932. A report is in progress on tabulated temperatures and departures by degrees of latitude along the Puerto Rico line, with comparisons of monthly mean sea temperatures and departures with corresponding temperatures at land stations.

EXPERIMENTS ON THE CULTURE OF DIATOMS, AND STUDIES OF THE YOUNG STAGES OF COPEPODS

M. H. CAMPBELL

I. Culture of Diatoms

The culture of a food supply for planktonic animals in the laboratory was attempted during the summer of 1932 in association with Dr. George L. Clarke.

The most abundant and healthiest growth of the diatom *Nitzschia closterium*, chosen for experiment, was obtained when the culture medium recommended by Dr. H. H. Gran was used. This is a modification of that employed by Dr. Allen in the Plymouth Laboratory, its formula being as follows:

1000 cc. sea water (sterilized by heating at 70°C. for 20 minutes).
10 cc.—.1% KNO_3
10 cc.—.1% Na_2HPO_4
5–10 cc.—soil extract.

No "pure," i.e. "bacterial free" cultures of *Nitzschia closterium* were obtained, but "persistent" cultures have been established to be used as food in cultural experiments with the copepod *Centropages typicus* Kröyer.

II. A Comparison of the Developmental Stages of *Euchaeta norvegica* Boeck, with those of *Euchaeta japonica* Marukawa

The six copepodid stages of *Euchaeta norvegica*, identified from plankton hauls from the Gulf of Maine, closely resemble the corresponding stages of the Pacific species *E. japonica*, but are distinguishable from the latter by the presence of an additional spine on the outer margin of the first joint of the outer ramus of the first swimming leg. Adult females of the two species differ in size and in the lateral projections of the thorax, adult males in the structure of the fifth leg.

III. Vertical Distribution of Developmental Stages of *Euchaeta norvegica*

A quantitative study was made of the occurrence of these stages in plankton hauls from various depths in the Gulf of Maine. The youngest stages were found in the deeper, never in the shallower samples, indicating that the species breeds in the deeper layers. These results are in agreement with Störmer's observations on this same species, as well as with the vertical distribution of *E. japonica* in the Strait of Georgia.

VERTICAL DISTRIBUTION AND DIURNAL MIGRATIONS OF PLANKTON IN RELATION TO THE PENETRATION OF LIGHT

GEORGE L. CLARKE

1. Collections were made over periods of 24 to 48 hours at 3 stations in the Gulf of Maine to study diurnal migrations down to a depth of 100 meters.

2. Measurements of the intensity of daylight falling on deck and penetrating to depths down to 53 meters beneath the sea surface were made at frequent intervals during these stations.*

3. Detailed analysis of the plankton hauls has been completed. The catches were divided into species, sexes, and stages of growth. The numbers of each of these groups taken at the different depths and times of day were estimated and correlated with the submarine illumination.

4. The photometers have been standardized against a 1000-watt Mazda standard lamp for which the spectral emission had been determined. The spectral sensitivity of the photo-electric cells was determined and the range of sensitivity applied to the standardization.

5. Simultaneous measurements of submarine illumination and the photosynthetic activity of pelagic diatoms were made in Woods Hole harbor at 3-meter intervals from the surface to the bottom. This work was performed in conjunction with Dr. H. H. Gran.

6. Development of methods for culturing copepods and diatoms in the laboratory was begun. It is hoped to test the physiological activity and reactions of these organisms under controlled conditions.

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* The addition to the submarine photometer of an internal shutter operated electrically and of a new four-conductor cable permitted observations to be continued even in rough weather.

INVESTIGATION OF PHYTOPLANKTON

DR. H. H. GRAN

Dr. H. H. Gran, visiting the laboratory at the invitation of the Institution, carried out an intensive quantitative survey of the horizontal and vertical distribution of phytoplankton in the parts of the Gulf of Maine, and of Georges Bank visited by the "Atlantis" in August. The procedure followed was the collection of known volumes of water at each station at several depths, from the surface downward, which were subsequently centrifuged in the laboratory, and the planktonic content counted. Dr. Gran also made a series of experiments on the influence of soluble organic compounds of iron on cultures of planktonic diatoms, by the method he has used previously on the Norwegian coast, and arranged a preliminary experiment on the relation of marine bacteria to planktonic diatoms, in collaboration with Dr. Waksman and Dr. Reuszer.

Before leaving Woods Hole Dr. Gran completed a paper on the results of these investigations, which is now in press, in the Biological Bulletin.

THE DISTRIBUTION OF TEMPERATURE AND SALINITY IN THE WESTERN NORTH ATLANTIC

COLUMBUS ISELIN

I. Since January 1st, 1932 the "Atlantis" has made 8 cruises, totalling 162 working days, on which temperature and salinity observations were made. During this time, data were secured from 125 deep stations and from 190 stations over the continental shelf. Work in the laboratory has been interrupted by 97 days at sea.

II. The reduction of the observations to a suitable form has involved:

a) The correction and checking of about 500 protected thermometer readings.

b) The calculation of depth by 500 unprotected thermometer readings.

c) The titration for chlorine of about 3000 water samples.

d) The recording of corrected depths, true temperatures and salinities on cards.

e) The construction of curves for T, S, σ_t, and $T-S$ ratio for all the deep stations.

f) The drawing of 9 temperature profiles and similar representations of the distribution of salinity and density.

III. My own time has been partly taken up in the above work, but I have progressed far enough in the examination of the material to have most of the diagrams and about half the manuscript ready for a preliminary report which will cover:

a) The distribution of temperature and salinity along the Bermuda sections and a study of the seasonal variations on the southern of the two lines.

b) The "Atlantis" observations across the North Atlantic Drift, the northern Equatorial Current, and the Antilles Current.

c) A study of the spreading at mid-depths into the Sargasso Sea region of water from the Mediterranean and from the South Atlantic.

STUDIES OF THE CHEMISTRY OF SEA WATER

NORRIS W. RAKESTRAW

Several chemical projects were undertaken in 1932, with the following results:

1. A more comprehensive study of the place and significance of nitrites in the nitrogen cycle in the sea was begun. Nitrite determinations were made on water samples from more than fifty stations, between New York and Portland, sometimes in conjunction with simul-

taneous determinations of nitrate. The effect of filtering and of long standing of samples was also ascertained. Nitrite and nitrate were determined in a few samples of water directly in equilibrium with the bottom. These experiments will be extended further, in view of important bacterial changes shown to take place in the bottom mud. The normal variations in nitrite were ascertained over a twelve-hour tidal cycle at one station in Buzzards Bay. Nitrite and nitrate at nine stations in the Gulf of Maine and on Georges Bank were correlated with phosphate, dissolved oxygen, and the results of phytoplankton and bacteriological investigations. The collection and publication of these general results have been delayed, awaiting similar determinations to be made during the winter months, for comparison.

2. A preliminary, but fairly extensive study of the occurrence of arsenic in sea-water was made. Total arsenic was determined by the best available method in the water from twenty stations, including two deep-sea stations on the line to Bermuda. Although the method used did not prove to be as accurate as was expected, still the results yielded a satisfactory measure of the normal amount of arsenic in sea water and established the fact that it is not subject to the regular, vertical variations characteristic of the compounds of phosphorus and nitrogen.

3. Methods for determining copper and iron in sea water were examined critically and the possibility revealed of improving the determination of the latter metal.

4. A substantial beginning was made on a method for the positive determination of dissolved nitrogen in sea water.

5. Some preliminary work was done on the adaptation of quantitative spectrographic methods to the analysis of sea water.

6. (By P. H. Mitchell) Improvements were made in one of the methods for determining the "buffer capacity" (alkalinity) of sea water. With the experience and data now at hand we can hope to make such measurements with a greater accuracy than heretofore. Preliminary results seem to show a remarkable constancy in this property.

INVESTIGATIONS BY THE PASSAMAQUODDY COMMISSION

On October 1, 1932 the following members of the staff of the International Passamaquoddy Fisheries Commission transferred from their summer laboratory at St. Andrews, New Brunswick, to the Oceanographic Institution at Woods Hole for the winter: Dr. E. E. Watson, Dr. C. J. Fish, Mr. Trygve Braarud, Dr. M. W. Johnson, and Miss H. E. Rigby, clerk. Dr. H. H. Gran, and Mr. M. Graham, returned to Norway and Great Britain respectively where they will complete their reports.

Field work in the Bay of Fundy and Gulf of Maine terminated in October and the staff will devote the remainder of the year in laboratory work and preparing a report to be submitted to the International Commission.

The object of this investigation has been to determine the existing hydrographical, chemical and biological conditions in the Fundy Region with a view to estimating what effect the proposed Cooper tidal power project might have on these conditions and the resulting effect on the fisheries.

STUDIES IN MARINE BIOLOGY AND BIOLOGICAL CHEMISTRY

ALFRED C. REDFIELD

During the summer of 1932 work was continued on the equilibrium of oxygen with the respiratory proteins of marine animals. Complete series of data were obtained for the blood of the squid at various carbon dioxide pressures and temperatures ranging from 5°C. to 25°C.

Studies have been made of the oxygen dissociation curves of the hemocyanin of the squid when diluted with various solutions. These studies, together with others previously made, form the basis for an extensive paper now in preparation on the comparative aspects of the equilibrium of bloods containing hemocyanin with oxygen.

Mr. Eric Fries was engaged in studies of the asphyxiation of the squid at various temperatures, which may be interpreted in the light of the data obtained upon the physical chemistry of the blood.

The capture of two porpoises by the collecting crew of the Marine Biological Laboratory has afforded an opportunity to study for the first time certain properties of the blood of a marine cetacean important in respiration. An account of this investigation written by Dr. Arda A. Green and myself has been published.

Dr. Raymond W. Root and Dr. Arda A. Green completed during the summer a study of the oxygen dissociation curves of the blood of the toadfish, *Opsanus tau* (Linnaeus), tautog, *Tautoga onitis* Linnaeus, and goosefish, *Lophius piscatorius* Linnaeus. Their research throws new light on the nature of the equilibrium of hemoglobin with oxygen. The report of this investigation is in press.

Mr. Frank A. Brown investigated the carotinoid pigments of Crustacea. He found that two pigments may be extracted from the shells of *Carcinides maenas*, *Homarus americanus*, and *Palaemonetes vulgaris*. One of these appears to be similar to plant carotin; the other is identical with the red pigment found in the blood of the lobster and in the shells of deep sea prawns. Experiments showed that the quantity of these pigments in the shrimps may be varied by keeping the animals under various conditions of illumination. Previously such color changes have been explained solely as due to contraction and expansion of the chromatophores.

My laboratory has continued to co-operate with Professor J. B. Conant of Harvard, Professor J. Barcroft of Cambridge University, and Professor E. Philippi of the University of Innsbruck, in supplying material for studies on the chemistry of hemocyanin which they are conducting.

During a short cruise on the "Atlantis" to the Gulf of Maine I made observations with a view to determining whether there existed diurnal alterations in the hydrogen ion concentration and oxygen content of the sea water occasioned by the interruption of photosynthetic activity during the night. No such effects could be established. Observations on the chemical constituents of the water occurring at various depths in the western basin of the Gulf of Maine showed, however, that the deeper waters have been considerably modified by biological agencies. The oxygen content below one hundred meters is only about three-fifths that observed at the surface. The water down to a depth of ten meters appears at this time of year to be in complete equilibrium with the oxygen of the air. That at twenty-five meters is supersaturated with oxygen, presumably as the result of photosynthetic activity. The water in the deeper layers also contains more phosphate and nitrate and is more acid than that of the surface whereas the nitrites display a zone of maximal concentration at a depth of fifty or sixty meters. It is believed that a further study of these conditions as they occur at different regions and at different times of the year may lead on the one hand to more definite information on the rates at which organisms modify the composition of sea water and on the other hand afford a method whereby the rate of circulation of the water in the Gulf of Maine can be determined.

INVESTIGATIONS IN METEOROLOGY AND PHYSICAL OCEANOGRAPHY

C. G. ROSSBY

A theoretical study of atmospheric and oceanic turbulence has been completed, and is now in print. The main result is a definite relation between surface wind, surface drift and the depth of the homogeneous (turbulent) layer in the ocean. Certain reasonably satisfactory checks have been obtained from previously collected data; additional checks are being made with the aid of about 500 pilot balloon observations; about 200 of these were collected by Mr. Lichtblau and myself on board the "Albatross II" and the "Atlantis," the remainder were made for us on Sable Island by the Canadian Government.

About ten months' evaporation records from the Nantucket Lightship and from the "Atlantis" are being studied. Additional evaporation measurements are in progress. Dr. Karl O. Lange has aided in experimentation in the development of apparatus for measuring the water transport in successive strata below the surface of the sea.

A seminar on boundary problems (heat and moisture transfer at ocean surface) was held three times a week, during part of the summer.

INVESTIGATION OF THE PHOSPHORUS CYCLE AND ASSOCIATED FACTORS IN THE NORTH ATLANTIC BASIN

H. R. SEIWELL ASSISTED BY GLADYS SEIWELL

Observations on the phosphorus cycle of the western North Atlantic (begun in 1931) were continued on the cruises of "Atlantis" in 1932. We now have observations on seasonal variations in the phosphorus cycle and associated factors in the open ocean on sections between the Bermuda Islands and the North American coast, between Bermuda and the Nova Scotia coast and between Bermuda and Chesapeake Bay. Observations on diurnal variation of oxygen and pH have been made at one station approximately fifty miles south of the Bermudas. Seasonal variations have also been studied in the waters on the coastal shelf between Chesapeake Bay and Cape Cod.

In general the observational program has consisted of determination of soluble phosphoric acid, total phosphorus, oxygen and pH at stations and depths wherever practical. Ultrafiltrations were made of fresh samples of sea water from all depths and the water treated for phosphorus analyses. The data are now being prepared and reduced for discussion.

PHOSPHORUS OBSERVATIONS

The determinations of soluble phosphoric acid on the major cruises of the "Atlantis" were all carried out in a special three tube colorimeter. Using this colorimeter it was possible, not only to compare the sample with the appropriate standard, but also permitted a rapid intercomparison of the samples. In this way, the errors in the analyses were reduced to a minimum. A further attachment to the colorimeter makes possible the analyses of water samples which are discolored by unusually large quantities of plants or debris.

In addition to determining the soluble or nutrient phosphorus in the sea, determinations of the total phosphorus were made on more than 500 samples from the "Atlantis" sections. This determination cannot be carried out conveniently at sea, thus the necessity of storing the samples until laboratory facilities are available.

H-Ion Observations

The determination of H-ion concentration of sea water was made on a double wedge comparator specially constructed for use at sea. The instrument was calibrated against known buffers over a wide working temperature range. Special precautions were taken to obtain specific temperature of every reading so as to permit the reduction of relative values to absolute values of pH.

A detailed study has been made of the vertical distribution of oxygen along the 40th meridian between 35° N. latitude and the equator as determined at "Atlantis" stations in the winter of 1932.

Interpretation of northerly and southerly components of movements of water masses has been attempted, based on maxima and minima of oxygen: also tentative calculation of the amount of oxygen consumed per unit per year. A report on this subject is now well advanced.

Chemical Analyses of Marine Plankton

During the 1932 cruises of the "Atlantis" eleven plankton samples from various depths were collected and subjected to organic carbon, total phosphorus, moisture and ash analyses.

Investigation of the Phosphorus Cycle in Marine Sediments

This problem begun in the summer of 1931 was continued with certain modifications in the summer of 1932 by Gladys Seiwell.

In the 1931 investigation a study was made of the phosphorus and organic matter contents of sediments in relation to their physical properties.

In the summer of 1932 a more detailed study of the organic matter, organic carbon, total and soluble phosphorus contents of sediments was carried out, in the hope of explaining the results obtained in 1931.

It appears from the results that both chemical and physical properties of shallow water sediments are to a great extent dependent on the conditions of sedimentation arising from the hydrographic characteristics of the region. It is for this reason, perhaps, that we find more or less well marked regional differences of characteristics.

The soluble PO_4 content of sediments was found to vary from approximately 0.15 mg. per kg. of sediment to more than 6.5 mg. per kg. of sediment. However, within individual regions we find less variation as shown by the preliminary results from 1932.

In general the H_2O_2 oxidizable organic matter has been found to vary as the colloidal ($>2u$) content of sediments.

The percentage of organic carbon and total phosphorus was also determined for a wide variety of sediments.

INVESTIGATION OF THE SEDIMENTS OF THE CONTINENTAL SHELF AND THEIR ENVIRONMENTS

In charge of H. C. STETSON

The methods of investigation, developed in 1931 were continued, in 1932, along traverses from the shore to the edge of the continent at the following places, samples being taken approximately every mile: Martha's Vineyard, Block Island, and Ocean City, Md., with three shorter traverses, one off Atlantic City, N. J., and two off the outer side of Cape Cod.

Mechanical analysis yields the following results:

1. There is a tendency towards progressive decrease of grade size from shore to the break slope although subject to violent fluctuations.
2. Degree of sorting bears no constant relationship to present depth of water.
3. Silt and clay usually appear in small quantities in 140–180 feet of water and gradually increase in amount, though they are, in most cases, the smallest part of the sample.
4. The topographic profile is probably not a profile of equilibrium for present sea level, particularly in its middle and outer parts, and has no influence on grade size.
5. The sediments on outer parts of the shelf are mixtures of material accumulated under at least two different environments. Deposition resulting from present sea level has not obliterated traces of shallower conditions.
6. An area of sand, equalling the St. Peter sandstone in degree

of rounding and with many frosted grains is present 20–45 miles off Martha's Vineyard, suggesting an ancient beach with sand dunes.

7. The lines off Nauset, Martha's Vineyard and Maryland, though of different lengths, are remarkably similar and show several major trends in common. First, a belt of coarse, red sand at 28–30 metres, second an abrupt change to an area of fine, well-sorted, gray sand mixed with silt and clay, and third, in the case of the line off Martha's Vineyard and Maryland a marked coarsening of grade size at the break in slope and for some distance further down. In the case of the Nauset line this coarsening occurs at about 170 metres. Except for the inner belt of coarse sand, the other features occur at progressively greater depths as we go northeastwards.

8. Several short lines from the Gulf of Maine are being worked at present, in the attempt to tie in this region with the shelf south of Cape Cod.

9. Experiments on transportation and deposition have been continued with sedimentation pans but have met with indifferent success, largely due to mechanical difficulties.

It is obvious that the surface sediments of the shelf are not all of them the result of conditions depending on present sea level. Where to draw the line between those for which the present environment is responsible and those left over from the Pleistocene, or even some Tertiary stand of the sea, is a problem that cannot be answered until more data have accumulated.

10. The heavy minerals were determined by A. E. Alexander, in about 150 of the samples, by the Larsen immersion method; their regional distribution shows the mineralogy of the continental shelf sediments to be almost identical throughout its length and breadth, with few exceptions. Concentrations of limonitic pellets were found off Martha's Vineyard and Block Island, and a detailed comparison made with limonitic material collected along the coast, leading to the conclusion that the limonite in the sea had been formed in place. Detailed examination has also been made of the occurrence of glauconite, found on every line where samples were taken, and of iron sulfide bodies.

REPORT OF STUDIES IN MARINE BACTERIOLOGY

SELMAN A. WAKSMAN, H. W. REUSZER, C. L. CAREY,
M. HOTCHKISS, ASSISTED BY C. E. RENN

The investigations in the field of marine bacteriology inaugurated in 1931 were centered in 1932 largely around four general problems: 1. the abundance and distribution of the bacterial population in the sea water and bottom deposits of the Gulf of Maine, the continental shelf and certain bays about Cape Cod, 2. decomposition of zoöplankton and algal residues by bacteria, 3. the rôle of bacteria in the nitrogen cycle in the sea, 4. the abundance and chemical nature of marine humus. The results can be summarized as follows:

The number of bacteria found in sea water, only a short distance away from land and at a depth which is not affected by the agitation of the bottom by currents and by wave motion, is comparatively small. Along the shores receiving considerable land drainage, much greater numbers of bacteria are found. Sea water in shallow regions (Georges Bank) contains larger numbers of bacteria than in the deeper waters (Gulf of Maine). There seems to be a definite relation between the nature and abundance of the plankton and the abundance of bacteria in the water. The sea bottom contains much larger numbers of bacteria than the sea water, mud bottoms more than sand bottoms. The activities of the bacteria are largely concentrated in the very uppermost layer of the bottom material; the numbers then diminish rapidly with depth. A definite parallelism was found between the organic matter content of the mud and the abundance of the bacteria.

In the study of the decomposition of marine residues by bacteria, the nature of the medium was found to be of considerable importance. The zoöplankton was decomposed more rapidly in the mud medium than in the sea water medium, as measured by the evolution of carbon dioxide; however, the amount of nitrogen liberated as ammonia was the same in both media. Probably the nitrogenous constituents of the plankton are decomposed alike in the water and in the mud, while some of the non-nitrogenous complexes decomposed more rapidly in the latter, due to the presence of specific bacteria. This phenomenon was emphasized particularly in the study of decomposition of Ulva and Fucus material by bacteria in sea water and in mud media. The Ulva with the higher nitrogen content decomposed alike in both media, but very little nitrogen was liberated as ammonia. The Fucus with a lower nitrogen content hardly decomposed at all in the water but decomposed well in the mud. The addition of a little available nitrogen, in the form of ammonium salt or nitrate, hastened considerably the decomposition of the Fucus in the water.

Among the bacteria concerned in the nitrogen cycle in the sea, three groups have been studied in detail, namely, 1. nitrogen-fixing, 2. nitrifying, and 3. denitrifying. The aerobic Azotobacter and anaerobic Clostridium were found to be present in the sea and capable of fixing appreciable quantities of nitrogen, provided an available source of energy was present. Nitrifying bacteria are absent in the water, but are present in the sea bottom, where active nitrification takes place. The nitrite-forming bacteria develop on artificial media more rapidly than the nitrate-forming organisms. Nitrate-reducing bacteria were found abundantly in the sea; however, denitrifying bacteria or those organisms which are capable of reducing nitrate to atmospheric nitrogen occur in the sea but seldom, largely in the marine bottom. These observations have an important bearing upon the cycle of nitrogen in the sea.

Several bacteria were isolated from the sea that proved capable of decomposing agar and cellulose. A detailed study has been made of the ability of these organisms to decompose carbohydrates under marine conditions and their importance in the decomposition of plant residue in the sea.

The occurrence and chemical nature of marine humus were studied in detail. The amount of humus in the marine bottom was calculated by multiplying the organic carbon content by the factor 1.923. The organic carbon was most conveniently determined by a modification of Schollenberger's method. The amount of humus in the marine bottom was found to vary considerably from a fraction of a per cent in sand bottoms and in deep ocean bottoms to 4–10 per cent in muds. The humus content of the mud usually diminishes with depth; in some cases, however, it was found to increase. The ratio of carbon to nitrogen in the marine humus was found to be more or less constant, varying from 8 to 12. A study has further been made of the chemical nature of marine humus and its relation to the chemical composition of marine plant and animal residue.

STUDIES ON (1) LIGHT AND THE BEHAVIOR OF MARINE ORGANISMS; (2) BIOLOGY OF PINNOTHERES MACULATUS SAY

J. H. WELSH

(1) During the summer of 1932 a survey was made of the effect of intensity of illumination on the behavior (especially rate of locomotion) of a number of marine invertebrates. Particular attention was given to comparing the behavior of animals which move principally by means of cilia with that of animals which move by means of muscles. Included in the survey were flatworms, annelid larvae, molluscan larvae and adults, and crustacean larvae and adults.

(2) Observations were continued on the biology of *Pinnotheres maculatus* Say and additional data were obtained on sex ratio, sexual dimorphism, and development.

List of Contributions

LIST OF CONTRIBUTIONS
FROM THE WOODS HOLE OCEANOGRAPHIC INSTITUTION

No. 1. H. U. Sverdrup. Scientific Results of the *Nautilus* Expedition, 1931, under the Command of Captain Sir Hubert Wilkins. I. Introduction and Narrative. *Papers in Physical Oceanography and Meteorology*, Vol. II, No. 1, pp. 3-15.

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No. 4. B. Villinger. Scientific Results of the *Nautilus* Expedition, 1931, under the Command of Captain Sir Hubert Wilkins. IV. Die Schweremessungen auf der *Nautilus* Expedition, 1931. *Papers in Physical Oceanography and Meteorology*, Vol. II, No. 3, pp. 3-16.

No. 5. Henry C. Stetson. Scientific Results of the *Nautilus* Expedition, 1931, under the Command of Captain Sir Hubert Wilkins. V. The Bottom Deposits. *Papers in Physical Oceanography and Meteorology*, Vol. II, No. 3, pp. 17-37. 1933.

No. 6. Selman A. Waksman and Cornelia L. Carey. Rôle of Bacteria in the Decomposition of Plant and Animal Residues in the Ocean. *Proc. Soc. Exper. Biol. and Med.*, Vol. XXX, pp. 526-527. 1933.

No. 7. Arda A. Green and Raymond W. Root. The Equilibrium between Hemoglobin and Oxygen in the Blood of certain Fishes. *Biol. Bull.*, Vol. LXIV, pp. 383-404. 1933.

No. 8. Norris W. Rakestraw. Studies on the Biology and Chemistry of the Gulf of Maine. I. Chemistry of the Waters of the Gulf of Maine in August, 1932. *Biol. Bull.*, Vol. LXIV, pp. 149-158. 1933.

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